Volume LXXXIV Number 4

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THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, London
THE MARUZEN-KABUSHIKI-KAISHA, Tokyo, Osaka, Kyoto, Fukuoka, Sendai
THE COMMERCIAL PRESS, LIMITED, Shanghai

Volume LXXXIV Number 4

THE BOTANICAL GAZETTE

A JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

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Issued December 23, 1927

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, Canary Islands, El Salvador, Argentina, Bolivia, Brazil, Colombia, Chile, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, Peru, Hayti, Uruguay, Paraguay, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Balearic Islands, and Spain. ¶Postage is charged extra as follows: For Canada and Newfoundland, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, £2 8*s.* each; single copies, including postage, 6*s.* 8*d.* each.

For China: The Commercial Press, Limited, Paoshon Road, Shanghai. Yearly subscriptions, including postage, $9.60; single copies, including postage, $1.33, or their equivalents in Chinese money.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

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Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March, 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

PRINTED IN THE U.S.A.

The PRESS IMPRINT

November and December 1927

A New Old Testament

As advance accounts in the newspapers some time ago intimated, J. M. Powis Smith of the University of Chicago, Theophile J. Meek of the University of Toronto, Alex. R. Gordon of the United Theological College and McGill University, and Leroy Waterman of the University of Michigan, have completed their American Translation of the Old Testament. The excitement that the new version has caused in the daily press recalls that brought forth by the appearance of the Goodspeed translation of the New Testament four years ago.

While controversy rages the translators let the matter rest as stated in their Preface to the translation:

"Why should anyone make a new English version of the Old Testament? With the Authorized Version of King James and the British and American revisions, to say nothing of unofficial renderings, have we not enough? This question may quite fairly be asked. The only possible basis for a satisfactory answer must be either in a better knowledge of Hebrew, or in a fuller appreciation of fundamental textual problems, or in a clearer recognition of poetic structures, or in such a change in our own language as would render the language of the older translations more or less unintelligible to the average man of our day. As a matter of fact, our answer must be found in all of these.

"The most urgent demand for a new translation comes from the field of Hebrew scholarship. The control of Hebrew vocabulary and syntax available to the scholar of today is vastly greater than that at the command of the translators of the Authorized Version, or of its revisers.

"Modern studies of textual problems reinforce the need for a new rendering. . . . . The science of textual criticism has made great progress in recent years, and no translation of the Old Testament can afford to ignore its results. Our guiding principle has been that the official Massoretic text must be adhered to as long as it made satisfactory sense. . . . .

"Much of the text that has long passed as prose is now recognized as really poetic both in form and spirit. This adds to the necessity for a new translation. Poetry should not be printed as prose. The present translation brings into clear light many of the hidden beauties of Hebrew poetry. . . . .

"The English of King James's day is not wholly natural or clear to the average man of the present day. . . . . The use of 'vinegar' in the sense of a wine or liquor for drinking has long since ceased to be recognized. . . . . Facts like these make the reading of the Bible a scholarly rather than a religious exercise, and clearly point to the need for a new translation. . . . .

"The content of the Old Testament is, with little exception, on a high literary plane. The language of the translation, therefore, cannot be allowed to fall to the level of the street. In this translation the foregoing principles have been kept clearly in mind. It tries to be American in the sense that the writings of Lincoln, Roosevelt, and Wilson are American . . . . it aims at being easily understood wherever English is spoken. . . . .

"The translators and the University of Chicago Press have sought to give this translation the appearance of a modern book. This purpose has determined the make-up of the page and has led to the addition of headings for paragraphs, and to the *insertion of half-titles before* individual books. It has also kept verse numbers out of the text and relegated them to the margin. . . . ."

In the best tradition of modern English writing this new version interprets the impressive, eloquent Hebrew of centuries ago.

The following passage from "The Song of Songs, Which Is Solomon's" is illustrative of those sections which the translators have cast into poetic form:

THE MAIDEN TO THE YOUTH

Kiss me with kisses from your mouth,
for your love is better than wine;
the fragrance of your ointments is sweet;
Your very self is a precious ointment;
therefore do the maidens love you.
Take me along with you, let us hasten;
bring me, O king, into your chamber,
That we may exult and rejoice in you,
that we may praise your love more than wine;
rightly are you loved.

And here is a part of the story of Ruth in the new translation:

RUTH'S APPEAL TO BOAZ

Then her mother-in-law Naomi said to her,

"Should I not be seeking a home for you, my daughter, where you may be comfortable? Now then, what about our relative Boaz, with whose women you have been? See, he is going to winnow barley at the threshing-floor tonight. Wash and anoint yourself therefore, put on your best clothes, and go down to the threshing-floor; but do not let your presence be known to the man until he has finished eating and drinking. See to it, however, when he lies down, that you note the place where he lies; then go in, uncover his feet, and lie down yourself; he will let you know what to do."

"I will do just as you say," she responded.

So she went down to the threshing-floor, and did just as her mother-in-law had instructed her. When Boaz had eaten and drunk, and felt content, he went to lie down at the end of the grain-heap. Then she came in stealthily, uncovered his feet, and lay down. At midnight the man started up, and turning over, discovered a woman lying at his feet!

"Who are you?" he said.

"I am Ruth, your maidservant," she said. "Take your maidservant in marriage; for you are a near relative."

"May the Lord bless you, my girl!" he said. "This last kindness of yours is lovelier than the first, in that you have not run after the young men, either poor or rich. And now, my girl, have no fear; I will do all that you ask; for all the counselors of my people know that you are a fine woman. . . . ."

THE OLD TESTAMENT: AN AMERICAN TRANSLATION. Edited by J. M. Powis Smith. *Cloth $7.50, leather $10.00, postage 25 cents extra.*

Silly? Crazy? Damnable Heresy?

Since the appearance of his *Jesus: A New Biography*, Shirley Jackson Case has been heralded from the Atlantic to the Pacific by newspaper reporters as the arch-heretic of the century. Dr. Case, in attempting to show the modern reader "not the Jesus of the stained-glass window, but the Jesus who lived and walked with men," has, it seems, done something exceedingly sensational. The feeling of the publishers about the matter, however, has been expressed by the Nashville *Presbyterian Advance*, a by no means radical magazine, better than they could do it themselves:

"It appears that Professor Shirley Case, who has published several excellent volumes, has written a book on the life of Jesus. It is not yet published. [It has since appeared.] Some newspaper writer, however, learned something about the book and wrote a sensational story affirming that in the new book Dr. Case declares that Jesus did not affirm his own divinity. Whether this is a fact or not remains to be seen, but on the basis of the assertion of the 'exclusive story' (unfortunately the word 'exclusive' in such cases has come to have about the meaning of 'lying' to discriminating readers) other reporters got busy and interviewed some ministers. These ministers, without having seen the book, promptly proceeded to call it 'crazy' and 'silly' and at least two of them are quoted as calling it 'damnable heresy.' If there is

anything 'crazy' or 'silly' about the whole business, it is to be found in those persons who so violently denounce what they know absolutely nothing about, except on the 'say-so' of some untrained reporter who is probably unable to distinguish between theological dogmas and historical facts."

Hasty and popular as they are, entirely oblivious to the unique methods of scholarship upon which Dr. Case's biography rests, the newspaper accounts have nevertheless shown one significant thing: that the public is intensely interested in new interpretations of Jesus' life and work. The modern reader is eager to look back over the intervening centuries to Jesus as he actually was.

Professor Case has long been known as a scholar who has studied, more fruitfully, perhaps, than any other, the social background of early Christianity. His method has been his most original contribution. It has enabled him to bring the reader of today into direct contact with the environment of beginning Christianity. It enabled him to dispel in his *Historicity of Jesus* the recurring hoaxes that treat Jesus' existence as a myth. And it has now made it possible for him to bring the reader closer than ever before to the real Jesus of history, as he appeared to the men of his own time in Palestine over 1900 years ago.

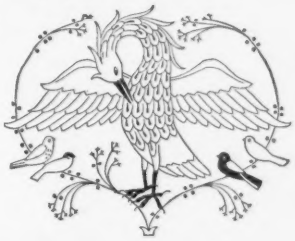
Within the past few years there have been many unhistorical and sentimental lives of Jesus, but there has not been a biography dealing in so thoroughgoing a fashion with the results of scientific investigation.

JESUS: A NEW BIOGRAPHY. By SHIRLEY JACKSON CASE. *$3.00, postpaid $3.15.*

* * *

The Ten Princes

Somewhere beyond the Himalaya Mountains lies Magadha, a land notorious for its unscrupulous delight in sensuous beauty and its ribald worldly wisdom. From this country, at a time when its people were gay, in love with poetry and pleasure, and shamelessly given to lying, fared Rajavahana and nine companion princes to conquer the four quarters of the universe. Dandin told the story of their adventures over a thousand years ago in *The Ten Princes*, his only prose novel. Arthur W. Ryder has retold it in a translation which retains the beauty of the original and adds a modern piquancy.



It seems that Rajavahana disappeared, and the nine companion princes, scattering to find him, met with a series of gay adventures in the course of which each gained a throne and an incredibly beautiful lady. Mr. Ryder's translation is a racy re-telling of Dandin's record, first of Rajavahana's experiences, and, as the other princes rejoin him, of their own exploits, related by them with appropriate flourishes and due exaggeration.

In these adventurers runs a pronounced strain of the picaresque. But rascals as they undoubtedly are, they must nevertheless be accredited with pleasant accomplishments. Handsome as no other mortals, erudite in ethics, astronomy, and metaphysics, they have a gift for the lute, are masters of magic, excel in horsemanship, and possess a scientific skill in thievery, gambling, and the arts of deception in general.

In their travels, they meet many beautiful maidens (versed in the arts of flirtation both major and minor), whom they charm by artifice and natural endowment, and describe in warm detail. And through their stories troop as great a crowd of shady characters, curious villains, and examples of shining virtues, as ever emerged from an oriental fancy: Buddhist nuns, unfaithful wives, sages, rakes, and kings, gay girls and gods, court ladies, merchants, thieves. Each has his appropriate philosophy which the princes remember to the last degree of subtlety, and readily report, whether the subject be the conduct of courtesans or the rules of political science.

Dandin was the master of a beautiful prose

style which has not lost in the translating. One feature of his art is sententious brevity. He looked upon prose as a more exacting medium than poetry itself. Of his life we know little more than that he flourished in India in the seventh Christian century. *THE TEN PRINCES.* Translated by ARTHUR W. RYDER. *$2.00, postpaid $2.10.*

Arthur W. Ryder has also translated from the Sanskrit *The Panchatantra* and *Gold's Gloom.* Achmed Abdulla says of *The Panchatantra:* "Fairytales. But Fairytales for grown-ups. Fairytales suffused with a wisdom that is ironic, slightly bitter, wire-drawn, perfectly civilized. Mr. Ryder's translation is most excellent. It shows not only a thorough knowledge of the subject but understanding, sympathy, style, and a keen sense of humor."

Gold's Gloom is bound in exceptionally attractive form, with bright-hued covers, and a specially designed title-page and headbands by Preissig. The quintessence of the wisdom and charm of *The Panchatantra,* it is a representative selection of some of the most captivating stories in the world. *THE PANCHATANTRA. $4.00, postpaid $4.15. GOLD'S GLOOM: TALES FROM THE PANCHATANTRA. $2.00, postpaid $2.10.* Translated from the Sanskrit by ARTHUR W. RYDER.

The World and Man

Critics unite in calling *The Nature of the World and of Man,* by Sixteen Ranking Scientists of the University of Chicago, one of the finest and most engrossing popularizations of current science in existence. A handsome new edition, with bright-hued covers, has just appeared. In accordance with its authors' wish that it should always represent the most recent knowledge of science, revisions have been made throughout the book.

Forest Ray Moulton, the astronomer, says of the volume's theme: "The Nature of the World and of Man is not only a great subject—in fact, the greatest one that human beings may investigate—but it is one rich in romance and filled with stirring adventure. It will satisfy, if anything can, the love of youth for heroic things. The giants of mythological days are far surpassed by the huge machines that are the untiring slaves of modern men. The eye of the fabled Cyclops was not even prophetic of the great telescope on Mount Wilson, the pupil of whose eye, so to speak, is 100 inches in diameter. Not all the magic of antiquity can match the marvels of any chemical laboratory. Physicians cast out demons by means of surgery, by the use of extracts of ductless glands, and by prescribing chemical compounds, and if they have not raised the dead even in a single instance, they have at least within a few decades increased the average span of human life by ten or fifteen years. . . . . In fact, reason and the laws of nature (mark well *reason* and the *laws of nature*) have become a sort of intellectual telescope, as it were, with which modern science looks back across the geological ages. . . . . " *THE NATURE OF THE WORLD AND OF MAN.* By SIXTEEN RANKING SCIENTISTS OF THE UNIVERSITY OF CHICAGO. *$5.00, postpaid $5.15.* (*Textbook Edition $4.00, postpaid $4.15.*)

More Contemporary Americans

In his second series of appraisals of American life and letters, Mr. Boynton gives the reader an unusual view of public taste in America, a view marked by absence of ridicule and obloquy, in short, a glimpse of what American culture has to show on the positive side. The design of the book centers around the theme that certain fine inherited traditions are surviving, that the public is not oblivious to them, and that a new public is developing with a taste distinctly its own.

Beginning with Melville, Bierce, and Hearn, men who wrote before their time and who belong in spirit to the present

day, Mr. Boynton proceeds to Hergesheimer, Anderson, and Lewis. Believing that it is as necessary for the critic to see and declare what the artist is trying to express as for him to discuss the mode of expression, he conducts the reader to the very center of each artist's own particular world.

Various aspects of the American scene from Paul Whiteman and the movies to the college insurgents appear in a lively discussion that ranges freely over the seven arts and over the traditions and innovations of American life. *MORE CONTEMPORARY AMERICANS.* By Percy Holmes Boynton. *$2.50, postpaid $2.60.*

⁂

Some Contemporary Americans, Mr. Boynton's previous book, still retains its popularity. In it he interprets the drama, the short story, and the poet's art from his background of American life and tradition, and gives his personal reactions to the new modes, forms, and men.

Sandburg, Dreiser, Mencken, Robinson, Frost, Lowell, Wharton, Tarkington, Cabell, Cather, O'Neill, and Bradford appear in this brilliant and engaging series of estimates of contemporary American literature. *SOME CONTEMPORARY AMERICANS.* By Percy Holmes Boynton. *$2.00, postpaid $2.10.*

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Chinese Painting

An extraordinarily beautiful volume with fifty-seven plates in collotype is *Chinese Painting* by John C. Ferguson. Chinese art as it really is and as it is valued by the country that fostered it is written into this book with authority and understanding.

Mr. Ferguson's qualifications for judging the art of China by Chinese standards—as it must be done—are many. For thirty-five years he has lived in China in close association with all the connoisseurs and critics of his day, who have been his guides in the examination of extant specimens of writing and painting.

Beginning with the earliest records of China, Mr. Ferguson has written the history of painting in that country to the eighteenth century. The introductory paragraph is characteristic of the spirit of the book:

"More than three hundred years ago Chang Ch'ou wrote in the Preface to his *Ch'ing-ho Shu Hua Fang* that he would like to be transferred into a bookworm, and promised that he would not injure any of the manuscripts or paintings in which he lived, but would be contented with mere existence in their company. Such was the delight with which one of the ablest critics of Chinese writings and paintings reveled in his enormous task of separating the good from the bad, the genuine from the false, among the accumulated ink-remains of previous generations. If Chang Ch'ou could have had his wish gratified by becoming a bookworm, he would not have been of that variety which Emerson placed in contrast with 'Man Thinking,' for he had a keen mind. His wish was the measure of his appreciation of the worthy writings and paintings that came under his observation. These were the products of man's soul and were quite unlike the work of man's hands in bronze or clay or jade. Through these writings and paintings Chang Ch'ou held communion with the spirits of the great artists and calligraphists who preceded him, and in their unseen world there were no limits of time or space." *CHINESE PAINTING.* By John C. Ferguson. *Boxed, $12.50, postpaid $12.75.*

⁂ ⁂ ⁂

Roosevelt

Turning aside from the hero-worship which has in the past been an almost unavoidable snare to biographers, however desirous of presenting Roosevelt as he actually was, Howard C. Hill has written a book which even at this late date shows Roosevelt in a new light.

The source material to which Mr. Hill has had access is more extensive than Roosevelt's biographers and historians have hitherto found available. Of this material, the most important part is the Roosevelt papers of the Library of Congress, to which only two or three others besides Mr. Hill have ever been given access. Although perhaps a quarter of his notes were censored by the official in charge, his book still includes the greatest amount of material that has ever been taken from this source.

Roosevelt the man, the creator of the Roosevelt myth, Mr. Hill would place before Roosevelt the statesman. Representative of Mr. Hill's view of Roosevelt in his official capacity is the following: "Rooseveltian imperialism as manifested in the Caribbean was opportunist in character, not planned or predetermined. Like the conscript fathers of ancient Rome, Roosevelt was led from one action to another by the swift current of events which during his administration seemed to change with kaleidoscopic rapidity. . . . . With each problem he dealt in the manner of a practical man of affairs rather than in the fashion of the doctrinaire or the man of predetermined policies."

The Boston Transcript comments, "We have Professor Hill to thank for a most interesting glimpse of our ex-President. . . . . He does not hammer away at his conclusions; he presents them gently. . . . . He invites us to strike a compromise, and therefore proves himself a most gracious gentleman, quite unlike any of his kind in the past or present. In his book we find for the student, an essay; for the interested reader, a narrative; for everyone, a striking portrait penned upon a background of living detail."

Harry Hansen in the *New York World* calls Mr. Hill's book "the sober second thought of the historian . . . . a very able study." And the *Herald-Tribune* says, "Professor Hill has turned the X-ray of historical criticism upon Roosevelt's Caribbean policy." *ROOSEVELT AND THE CARIBBEAN*. By HOWARD C. HILL. *$2.50, postpaid $2.60.*

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By

ELLIOT R. DOWNING

Scientific study of science teaching has had some valuable results, and these Mr. Downing summarizes in this volume. Here is shown how the growing body of facts about the pedagogy of science, which have been ascertained by the experimental method, may be applied practically to the problems of the science teacher.

This book describes the social and economic background of the science-teaching movement, the history of science teaching in our elementary and secondary schools, present conditions in science teaching, and the aims held.

From this point Mr. Downing takes up the principles of selection and organization of science materials, and actual methods of instruction—by book, lecture-demonstration, and laboratory. There is specific information on notebooks and drawings, field trips, testing results; and suggestions are made for a complete science library in the school.

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By Sixteen Members of the Faculty of the University of Chicago

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VOLUME LXXXIV NUMBER 4

THE
BOTANICAL GAZETTE

December 1927

ANATOMY OF LEAF OF BANANA, MUSA SAPIENTUM L. VAR. HORT. GROS MICHEL[1]

ALEXANDER F. SKUTCH

(WITH FORTY-FOUR FIGURES)

I. External features

1. DISTRIBUTION OF LEAVES ALONG STEM; PSEUDOSTEM

Until a certain stage in the development of the plant, the entire aerial portion of the banana is made up of leaves alone. To a superficial observer, the banana of this age appears to consist of an erect, unbranched stem surmounted by a crown of enormous leaves, to be, in fact, a "tree," as it is often loosely designated, with a "trunk" 3–4 m. high and 15 cm. or more in diameter. If, however, the "trunk" is cut across at any level above the base, it is found to be a pseudostem composed of the overlapping, close-fitting leaf sheaths alone, and containing no axial member at all. As the plant approaches maturity, the condensed stem at its base begins to elongate and to push upward in the center of the pseudostem, finally emerging at its upper extremity, where it bends downward and produces the pendent inflorescence. The leaf insertions, which previously were in contact on a stem condensed into a bulb, are more and more widely separated along the lengthening axis, and internodes of increasing length appear.

[1] Botanical contribution from the Johns Hopkins University, no. 88. This investigation was made and its results are published with the aid of a grant from the Department of Agricultural Research of the United Fruit Company.

Whether or not the banana has a true aerial stem is a question more of academic than of practical importance. As SCHUMANN (20) observes, the phyllotaxy, whether distichous and alternate or spiral with a smaller divergence, is of more importance in determining the appearance of a genus of the Musaceae than the degree of development of the axis. However, since the theoretical interpretation is apt so to color the mere statement of the actual situation that if it is a mistaken one a false impression will be left in the mind of the reader, it seems desirable to re-examine the facts. Most brief accounts of the structure of the banana plant, particularly the more or less popular ones (4, 18, etc.), place so much stress upon the peculiar pseudostem, contrasting it with the "true" stem or bulb at its base, and with the floral axis which pushes its way upward through the former toward the light, that an erroneous picture of the aerial stem is very naturally formed. One might well expect, after reading these accounts, to find the inflorescence borne on the end of a gigantic, leafless scape, naked except for the bracts just beneath the flowers. Accordingly, the writer was considerably surprised, when he made his first longitudinal section of an entire plant, slicing it up the center with a long cutlass, to find the stem leafy almost to the summit (table I). WITTMACK (22) gives a clear statement of the facts, but the writer was unable to obtain this article until later. The other accounts just referred to contain no actual misstatement of the case, but are at fault rather from lack of completeness, and in particular from false emphasis.

Table I gives the sizes and heights of insertion of all of the leaves present on two fruiting plants. In these plants, respectively 396 and 422 cm. high, the largest leaves were inserted 30–150 cm. above the ground, and the absolutely largest at 69 and 67 cm. respectively, while the stems bore true foliage leaves to about 3 m. above the base. In other words, more than the basal three-quarters of that portion of the stem within the leaf sheaths was leafy. WITTMACK records the heights of insertion of the leaves of a specimen of *M. ensete*, which resembles *M. sapientum* in the distribution of leaves along almost the entire inclosed portion of the stem. The sizes of the leaves are not recorded in his table, but the largest leaf scar, and presumably the largest leaf, occurred at 49 cm. from the ground. On the

other hand, the stem which bears these leaves is totally incapable of supporting them without the aid of the inclosing sheaths. Whether or not the elongated portion of the axis which bears the largest leaves is to be considered a true stem, as well as the condensed basal portion which bears most of the leaves, is a question of definitions. Morphologically it is certainly stem, whatever we may choose to

TABLE I

SIZE AND DISTRIBUTION ALONG STEM OF INTACT LEAVES OF TWO FRUITING PLANTS

Number of leaf	Plant I* Pseudostem = 396 cm. high			Plant II† Pseudostem = 422 cm. high		
	Height of insertion	Length of leaf	Greatest width	Height of insertion	Length of leaf	Greatest width
1	Basal	262 cm.	79 cm.	19 cm.	274 cm.	79 cm.
2	Basal	178+‡	86	22	274	86
3	8 cm.	279	84	24	282	Withered
4	9	292	84	28	277	89
5	14	284+‡	89	29	300	89
6	18	239+‡	86	36	310	91
7	25	302	97	43	323	91
8	32	305	94	53	315	91
9	43	330	99	67	328	84
10	69	330	97	98	318	84
11	109	320	99	146	300	84
12	180	305	86	224	272	75
13	277	226	74	318	178	56
14	363	Dead§	Dead§	387	Dead§	Dead§

* 15 sheaths, totally or partially alive, the laminae belonging to which had fallen away, were removed from the outside of the pseudostem.

† 16 sheaths were removed.

‡ End of lamina torn off.

§ "Protecting leaf."

call it in regard to function. The banana may well be likened to any of our familiar perennial herbs of field or garden, which during the first year form a basal rosette of leaves at the crown of the rhizome, and in the succeeding season produce a leafy, aerial, flowering stem; only in the banana, a tropical plant, there is no resting season, and the circumstance that the leaves of the rosette are erected into a pseudostem which conceals and supports the true aerial stem is apt to be confusing. Even the pseudostem is not without representatives among temperate plants. The leek furnishes a good but diminutive example, although here the flowering shoot is lateral and not cen-

tral, as in the banana. The celery plant forms a larger but rather loose pseudostem.[2]

2. Phyllotaxy

The phyllotaxy is not constant throughout the life of the plant, but the angle of separation between successive leaves increases with the age and size of the individual. The fraction expressing the spiral of phyllotaxy was determined both by counting the number of leaves and of turns between two superposed leaves on the pseudostem, and by studying the arrangement of the sheaths of cross-sectioned pseudostems. Consistent results were obtained by the two methods, except in the case of very young suckers, where, probably as a result of a slight torsion of the pseudostem, the leaves often show a divergence very close to 1/3, although the angular separation as determined by the sheaths is nearer 2/5. The actual separation of the sword leaves of a young "ratoon," 50 cm. high or less, is often very slightly over 1/3. Somewhat larger ratoons show very clearly a phyllotaxy of 2/5, both of the blades and of the sheaths. At a somewhat later stage the spiral becomes clearly 3/7, both in the blades and sheaths. The size of the plant when the transition occurs depends largely upon its situation. A ratoon growing up at the base of a large plant, where it is shaded and produces sword leaves for a long time, may retain the 2/5 arrangement until it is over 120 cm. high, while an isolated sucker planted in the open, where it soon forms broad leaves, may show a 3/7 phyllotaxy at less than half that height. The two arrangements may often be seen in the same plant, the divergence between the older (lower) leaves being 2/5, that between the younger (upper) 3/7. Mature plants always have a phyllotaxy of 4/9, which may occur in plants 2 m. high, and is very clearly maintained in plants bearing full bunches of fruit, the presence of the bent-over stem causing no confusing distortion of the spiral.

The angular separation of the leaves of a very young ratoon, then, is slightly more than 120°. At a somewhat later stage, the age and size of the plant depending upon the environment, it becomes 144°

[2] *Veratrum album*, among temperate monocotyledons, provides an excellent example of the pseudostem. See Mrs. Arber's *Monocotyledons*, fig. xxxiii (Cambridge, 1925).

(in the ideal case). Still later it increases to 154°, and in full grown plants and those bearing fruit, the angular divergence is 160°.

WITTMACK gives the phyllotaxy of *M. ensete* as 3/7. GREVE (8) repeats this statement, and adds that it is true for all species of *Musa*. In young seedlings he found the phyllotaxy to be 1/2. SCHUMANN (20) records the 3/7 spiral as general for the Musoideae. In addition to examining numerous examples of the Gros Michel variety on the plantation, the writer had the opportunity to inspect the varieties Apple, China, Honey, Ramkelat, Robusta, and White House, as well as "*Musa kewensis*," in Hope Gardens, Jamaica. Mature fruiting plants of all of these showed a divergence of 4/9, younger plants 3/7 or less, according to their age. A single fruiting plant of the slender-stemmed *M. kewensis* maintained the 3/7 divergence of its leaves to the last.

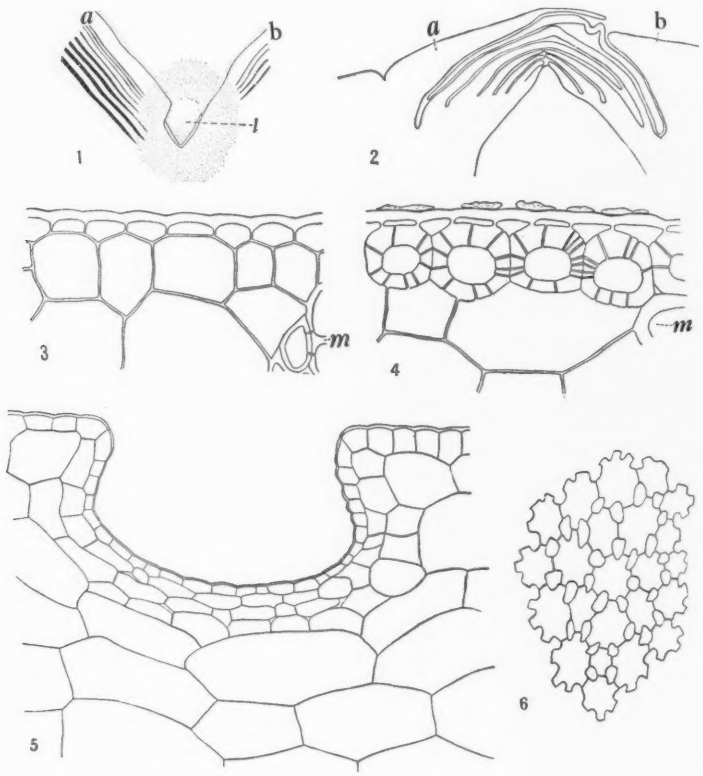
The spiral in which the leaves are arranged is always left-handed; it rises from the right to the left of an observer facing the stem.

At their insertion the leaf sheaths entirely surround the stem, the scar forming a complete circle. The insertion may be somewhat oblique, and in some of the higher leaves is markedly so. Here the vertical distance between the highest and lowest point of the scar amounted in one instance to 8.8 cm. where the stem was only 5 cm. in diameter. There seems to be no rule as to which point is uppermost; any point on the entire circumference of the scar may occupy that position. WITTMACK found that in *M. ensete* the scar does not completely surround the stem, but occupies 59–80 per cent of the circumference. He records even larger obliquities, amounting to 22 cm. The diameter of the stem is always considerably less just above the leaf insertion than just below it.

3. LATERAL BUDS

The lateral buds do not occur in the axils, as stated by SCHUMANN, but are situated opposite them. They regularly appear in the angle of the V formed by the two margins of the sheath as they converge to the point of insertion (fig. 1). The scarious margins overlie the outermost scales of the bud (fig. 2). Buds occur regularly at the insertion of each of the basal leaves of the plant, but not of those situated in the region of elongated internodes, that is, the

aerial stem. The position of the buds suggests that possibly the stem is a sympodium, but the point has not been investigated anatomi-



FIGS. 1–6.—Fig. 1, lateral bud (*L*) in surface view; *A*, *B*, margins of sheath of about 16th leaf from center of pseudostem; ×1. Fig. 2, horizontal section through similar bud; ×10. Fig. 3, outer epidermis and subjacent cells from a sheath near inside of pseudostem; *M*, portion of fibrous strand; ×400. Fig. 4, same but from exposed sheath of same plant; ×400. Fig. 5, pit from inner surface of sheath, in horizontal section; ×150. Fig. 6, stellate cells from transverse septum of sheath; ×36.

cally. In the related *Ravenala madagascariensis* the buds are truly axillary.

4. Leaf parts

Four regions along the length of the leaf are distinguished, but the divisions are suggested by convenience of treatment entirely without regard to morphological considerations, and the transition

Fig. 7.—A "protecting leaf" which hangs over a bunch of immature fruit

from one region to another is gradual and not sharp. These are, from the apex downward: the precursory appendage,[3] the lamina, the petiole, and the sheath. The appendage is a temporary organ, and has already withered when the leaf reaches maturity. The lamina contains a strong midrib which is flanked on either side by a pulvinar band. This is followed by a broad blade, bordered in the

[3] So I translate the *Vorläuferspitze* of German authors.

young leaf by a scarious margin which soon withers away. The petiole is a region of transition from the sheath to the midrib, in the larger leaves reaching 30 cm. or more in length. The sheath may be defined as that portion of the leaf which participates in the formation of the pseudostem.

5. Size and form of lamina

The lamina of the banana, in its pristine state as it emerges from the pseudostem, represents one of the largest unbroken expanses of photosynthetic tissue in the entire plant kingdom. In the largest leaves of the Gros Michel it attains a length of about 4 m. and a maximum breadth of over 1 m. It is exceeded in size notably by the enormous leaves of another member of the Musaceae, *Ravenala madagascariensis*, which are stated to attain 6 m. in length, by the floating leaves of *Victoria regia*, the largest of which reach 4 m. in diameter, and by the blades of certain kelps, such as *Laminaria longicruris*, attaining 6 m. in length and 1 m. in breadth. They are approached and perhaps equaled in area by the leaves of certain species of *Gunnera*. Of course, many tree ferns and palms have leaves of greater dimensions, but these are all compound, and not directly comparable with those of the banana. It is a significant fact, the bearing of which will be evident when we consider the splitting of the lamina by the wind, that the banana and its relatives bear perhaps the largest undivided aerial leaves to be found anywhere. Reliable data on the size of leaves are difficult to obtain.

FIG. 8.—Transverse section of leaf as rolled inside pseudostem, showing form of vernation; ×3.

The ovate-oblong leaves of the mature form are the last of a long series, the first of which are strikingly different in both size and shape. The first leaves arising from the subterranean bud, which grows out from the bulb to form the replacement shoot or ratoon, are reduced scale leaves, in which the portion that represents the sheath alone is prominent. In the succeeding leaves the lamina

becomes more and more conspicuous, first in the form of the long narrow "sword leaves" characteristic of the young ratoon, and as the plant grows older, gradually increasing in size and relative

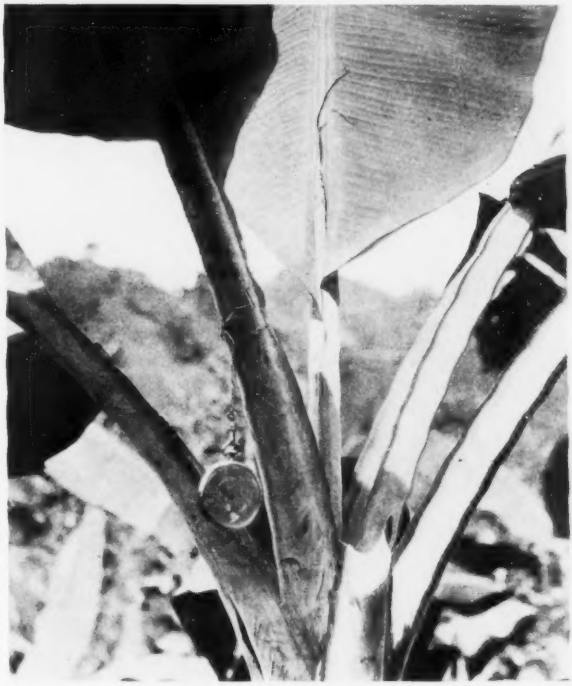


FIG. 9.—Young leaf just emerging from pseudostem; note long precursory appendage.

breadth until the mature proportions are attained.[4] The rapidity of change depends upon environmental conditions, of which light is probably the principal. Isolated suckers form broad leaves much

[4] According to COOK (3), there are two kinds of suckers, which differ in the character of their early leaves. The "broad-leafed" suckers originate from buds situated near or above the surface of the ground, and produce broad leaves from the first; the "sword" suckers arise from more deeply buried buds, and produce the characteristic linear leaves. For many excellent illustrations of leaves of both forms, and of the plant as a whole, see REYNOLDS (19).

earlier than those still attached to the parent plant. The fruiting plant retains about 11–14 intact leaves, together with about 12–16 sheaths which are still in part or entirely succulent and aid in the support of the stem, and perhaps serve as storage organs, although their blades have withered and fallen.

FIG. 10.—Leaf which has emerged from pseudostem to almost full length of lamina, but which has not yet begun to uncoil, at top of figure near middle.

6. "PROTECTING LEAF"

The last of the series of green vegetative leaves differs considerably from those just below it, and is distinguished as the "protecting leaf." In comparison with the other leaves it is very much reduced in length, but about equally broad (fig. 7). The broad flat

petiole retains a sheathlike structure, and clasps the inflorescence axis as it emerges from the top of the pseudostem, preventing more or less completely the penetration of rain water between the innermost sheaths and the stem. It is incapable of supporting the lamina, which bends forward and usually hangs for a time over the inflorescence, affording it temporarily a certain degree of protection from sun and rain. This leaf is inserted very near the top of the pseudostem, in the plants measured 30–40 cm. from its apex. It is short-lived, and droops and withers before the fruit is mature.

FIG. 11.—Dome which caps lamina just before it begins to uncoil, surmounted by partially withered precursory appendage.

7. VERNATION

In vernation the leaf is convolute. The rolled right[5] half of the lamina, making about 18 turns at its broadest portion, fits neatly into the concavity of the upper side of the midrib, and the whole is surrounded by the 4–5 turns of the left half (fig. 8). Preceded by the precursory appendage, the coiled leaf pushes upward from the center of the sheath of the one next in age (fig. 9), and emerges to practically the full length of the lamina as a straight, stiff, green rod (fig. 10). Then the coils begin to loosen, and the leaf first expands at the apex. The hollow cylinder which is formed just before the leaf spreads out terminates in a dome or cupola which is surmounted by the precursory appendage (fig. 11). The right half of the lamina alone participates in the formation of this dome, while the left half, rolled about the outside of the cylinder, may readily uncoil and spread out. Since the tissues of the dome undergo no

[5] As reckoned by an observer standing at the petiole and looking toward the apex of the leaf in its normal orientation.

further expansion, mechanical considerations make it at once evident that the right half cannot unroll without tearing away the permanently curved portions. The rupture of tissues occurs principally along the veins, and the dome is removed more or less intact. Usually the dome with the appendage remains at the end of the midrib, but sometimes they are torn from the latter and carried to

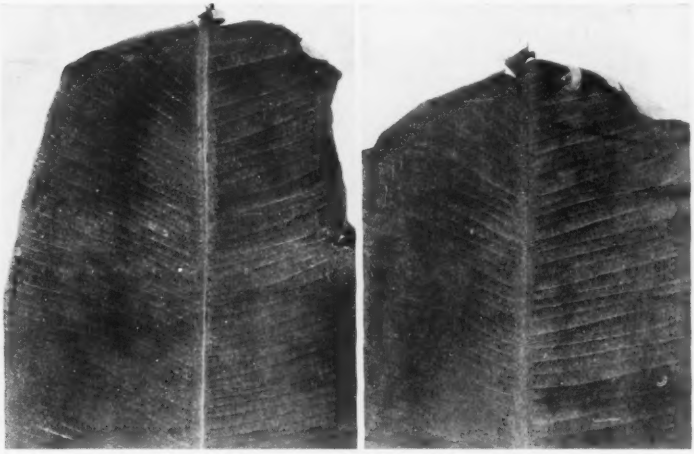


FIG. 12 FIG. 13

FIGS. 12, 13.—Apices of lamina of two leaves which have just uncoiled, showing torn right end and remains of dome and precursory appendage; in fig. 12 tear is straight and dome intact, in fig. 13 tear is more irregular.

the right side of the leaf. The line of separation is evident throughout the life of the leaf, and is more or less regular, according to whether the tear follows a single vein (fig. 12) or strikes across the lacunae from one vein to another (fig. 13).

II. Anatomy

A. SHEATH

8. GROSS STRUCTURE

The sheaths innermost at any particular stage of growth extend for practically the entire height of the pseudostem. Exception must be made, of course, for the leaves inserted along the late-appearing

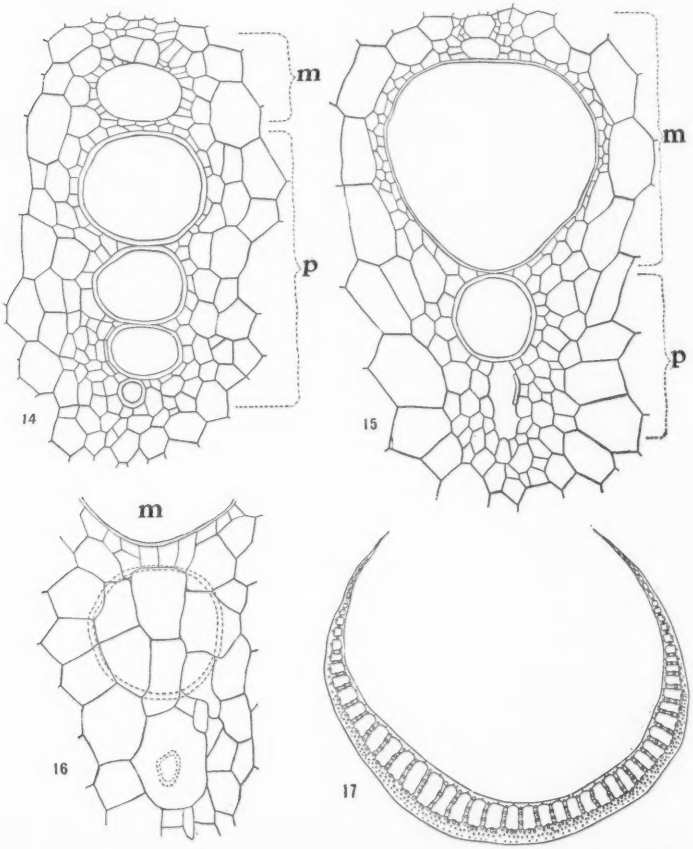
aerial shoot. Although the margins of the young sheath overlap and the sheath forms a hollow cylinder, the older, more exterior sheaths are typically crescentic in cross-section (fig. 17). The thickest point is near the center, whence the cross-section tapers to a thin transparent wing, which contracts at the margin to the thickness of two extremely narrow cells (about 9 μ). At the base the maximum thickness of the sheath is about 14–17 mm., at the top, about 10 mm. At its insertion on the stem the sheath completely surrounds it. It narrows abruptly to about half the initial breadth at a point 30 cm. higher up, whence it contracts gradually to the petiole. At their bases, old sheaths are penetrated while still alive by roots arising from that portion of the stem inclosed by them.

Between a thick outer and a thinner inner wall is situated a series of lacunae which occupy the bulk of the volume of the sheath. A series of radial septa runs the entire length of the sheath and divides the cavity between the walls into longitudinal canals. These are interrupted at frequent intervals by thin horizontal septa, with the result that a series of rectangular air chambers is formed. In the widest portion of large sheaths the lacunae are about 5–8 mm. wide (tangential dimension), 8–11 mm. deep (dimension normal to surface of sheath), and 2–6 mm. from top to bottom. At the base of the pseudostem the inner wall of the sheaths is often strongly corrugated, and the horizontal septa strikingly folded. This condition is reminiscent of the wavy grain of the wood at the base of large trees, and is a result of the same mechanical stresses in tissues of the same function but very different homology.

9. Dermal system

The epidermis is composed of straight-walled cells, rectangular in surface view, with their long axes parallel to the length of the sheath. Beneath the epidermis is a hypodermal layer of cells having their long axes parallel to those of the epidermis in the case of the outer surface, but perpendicular to them in the inner surface. Stomata occur on both the outer and inner surfaces of the sheath throughout its length, as well as on the inclosed portion of the stem even to its base. On the inner surface of all the sheaths and on the stem the stomata are not exposed to the atmosphere, since the

sheaths fit very tightly together. On the outer surface of some sheaths they are after a long period exposed, but here also on many



FIGS. 14–17.—Fig. 14, xylem portion of vascular bundle of sheath of leaf just emerging from pseudostem, section cut at about 5 mm. above insertion of sheath; protoxylem elements (*P*) still intact, metaxylem (*M*) immature; ×160. Fig. 15, bundle from sheath of about same age, section at 24 cm. above insertion of sheath; earliest protoxylem elements disrupted, and large metaxylem element almost mature; ×160. Fig. 16, portion of mature bundle, showing site of occluded protoxylem elements; annular thickening and turn of spiral band, slightly below plane of drawing, indicated by broken lines; ×285. Fig. 17, transverse section of sheath from fruiting plant, position of principal vascular bundles indicated by circles; ×about 0.5.

sheaths they are never brought into contact with the air. The presence of these stomata seems a result of phyletic inertia rather than physiological advantage. The average density of stomata on the outer surface was found to be 11 per mm.^{2} on the inner surface, 7 per mm.^{2} near the base, and 12 per mm.^{2} at the top. The density of stomata on the stem itself is about the same as on the sheaths. The stomata agree in general form with those described later for the lamina, but the cuticular ridges are more pronounced, the front and back cavities larger, and the substomatal chamber narrower and deeper. They are larger than those of the lamina, and those on the inner surface are larger than those of the outer surface, agreeing with the larger stomata on the upper rather than on the lower surface of the lamina. Length of stomata on inner surface is 34–38 μ, outer surface 27–34 μ.

The character of the epidermis does not vary greatly according to whether it is taken from a sheath near the center of the pseudostem, or from one which is exposed to the air. A very thin cuticle overlies a thick wall of cellulose (figs. 3, 4). In places a waxy layer is present over the cuticle. The cutinization of the guard cells resembles that to be described for the lamina. The epidermal layers of two sheaths in contact adhere so strongly that in stripping off a sheath it is a common thing to find that the thinner portions at the edge remain attached to the one within, and tear away from that which is removed.

The cells of the hypodermal layer of the outer surface show a profound difference in form and chemical composition, according to whether an outer exposed, or an inner protected sheath is taken (cf. figs. 3 and 4). If an inner sheath is selected, say one which is covered by four or five others, or any nearer the center than this, the hypodermal layer will be found to consist of cells with large clear lumina, and thin walls of unaltered cellulose; they show no trace of suberization. An entirely different picture is presented by the hypodermal cells of the outermost living sheath. The walls are much thickened, having attained 4–13 μ in thickness where in the inner sheath they are less than 1 μ. They are penetrated by prominent radial pits and are distinctly laminate in places. The lumina are very much reduced. Microchemical tests show that these thick

walls are lignified. The anticlinal and inner walls of the epidermal cells are thickened and lignified, while the outer walls and the cuticle are apparently unaltered. Occasionally the walls of the cells of the layer next beneath the hypodermal layer are somewhat thickened and suberized.

The alteration of the hypodermal cells begins when the sheath, which is pushed from the interior toward the exterior of the pseudostem by the dying off of the old sheaths on the outside and the pushing up of new ones within, is fourth or fifth from the surface. The walls thicken gradually and become suberized,[6] lignification not occurring until a later stage, when the sheath is covered by only two or three of those exterior to it. The hypodermal cells bordering the substomatal chambers behave differently from the others, which alone are referred to in this description, in that their walls thicken and become lignified at an earlier period. Thus, for example, in the fourth sheath from the exterior they were found to be greatly thickened (as much as the other cells in the second or even the exterior sheath) and lignified, while the surrounding cells were very slightly thickened, and the chemical alteration had proceeded only as far as a faint suberization.

Since the alteration of the hypodermal cells commences in sheaths still tightly covered by those exterior to them, it is obviously not caused by their proximity to a transpiring surface. The penetration of sunlight is probably the stimulus initiating the changes in a given sheath. The first indications of the thickening and suberization of the walls (except of the cells bordering the substomatal chambers) are usually to be found in that sheath which is the innermost containing visible traces of chlorophyll, and there is at least a roughly quantitative correlation between the greenness of a sheath and the thickness of its hypodermal walls.

The hypodermal stratum of the inner surface of even an outer-

[6] That is, they show a strong retention of GRÜBLER'S cyanin, stain yellow to brown with chloroiodide of zinc (with or without previous treatment in javelle water), are insoluble in conc. H_2SO_4, and do not stain in a solution of phloroglucin and HCl. Membranes are said to be lignified when they are insoluble in conc. H_2SO_4 and give a positive reaction with phloroglucin. They usually stain some shade of orange or brown in the chloro-iodide reagent. The same significance is attached to these terms throughout the remainder of this article.

most sheath is not greatly different from that of the inner sheaths. The walls of the former are faintly suberized, but not noticeably thickened, and never lignified. In this surface occur numerous small depressions or pits (fig. 5). These are circular to elliptical in outline, and vary greatly in size, the largest being 500 μ in diameter and 160 μ in depth. Their frequency is roughly 20–30 per cm.^{2} Both the epidermal and hypodermal cells beneath the pits are shallower than elsewhere, and small intercellular spaces containing air occur among the latter. The pit is lined with a cuticle perhaps slightly thicker than that found on the surrounding surface. When a section of the sheath, the cut ends coated with vaseline, is immersed in an aqueous solution of safranin or methylene blue, the stain penetrates and colors the whole inner surface, with the exception of the pits, which are impervious to it and remain white. The function of these pits, if any, remains problematical.

10. Vascular and mechanical systems

The ground tissue of the walls which border the lacunae of the sheath at their outer and inner ends and of the longitudinal septa is a thin-walled parenchyma, in which are distributed the vascular bundles and the strands of prosenchyma (fig. 17). The most prominent vascular bundles are those situated in the longitudinal septa. In the thicker portions of the sheath there are 2–4 large bundles in each septum, in addition to very few small ones, and occasional fibrous strands. A large bundle is situated in the outer wall opposite the center of each lacuna, and another where each septum joins this wall. Alternating with these is a series of smaller bundles, somewhat nearer the outer surface, and still a third series alternates with and is external to the second. Exterior to these are numerous small bundles, in which the vascular tissue is very much reduced or lacking, and the outermost bundles are reduced to mere strands of fibers. In the inner wall there are usually 1–3 small bundles opposite each lacuna, but the number may increase to 8 or 9 near the base of the sheath.

The development of the xylem, and in particular the fate of the protoxylem, present features of special interest and were studied in some detail. The course of the ascending sap in leaves of various

ages was traced by injecting the bundles with trypan blue in 1 per cent solution, as recommended by Miss BUCHHOLZ (**2**). Whole plants were cut from the bulb and set in the solution in a sunny place, where they were allowed to remain for six hours. In this period there was considerable upward movement of the dye, even through leaves still inclosed within the sheath and hence not transpiring, probably largely in response to the requirements of the tissues for growth.

The bundles at the inner edges of the longitudinal septa are those which show the largest series of protoxylem elements. The earliest and narrowest protoxylem elements have annular thickenings. Following upon these are tracheids in which annular thickenings may alternate with spiral bands. Next follow ducts thickened by a single spiral band, which in places may be doubled by splitting. Each of the successive protoxylem elements, of which there are 3–6 in the larger bundles, according to the bundle and the level of the cross-section, is of greater diameter than the last, until the single large metaxylem tracheid is reached. This tracheid is strengthened by 10–14 low-pitched spiral bands which divide and reunite, so that even in a single tracheid their number is not constant. This large element is succeeded toward the phloem by several narrow, spirally thickened tracheids which may briefly be designated the commissural connectives, since it is to them that the commissural tracheids are joined. The writer has distinguished as protoxylem all elements of temporary duration, and as metaxylem all which are permanently functional.

The spiral bands of these tracheids, like those of the tracheids in the stem, pull out readily, and a cutlass used in cutting up the banana plant (especially if it is not very sharp) is soon enveloped in a thick wad of them. WITTMACK quotes PACTON (**15**) as stating that this material once was employed as tinder in the West Indies.

As is generally known, the sheath of the banana leaf elongates through the activity of an intercalary growing zone situated in the region of its insertion on the stem. The rate of elongation of the leaf is very rapid, and MAXWELL (**13**) has recorded a maximum rate of growth of 21 cm. per day (see also TRELEASE **21**). A cross-section just above the base of a sheath which is still growing shows that

in each bundle the 3–5 earliest protoxylem elements are still intact and stained with the trypan blue, indicating that they are active in conduction (fig. 14); several more protoxylem elements are in process of formation, and the large metaxylem tracheid is still small and unthickened. At a higher level the rings of thickening of these earliest elements have become more widely separated, and the surrounding parenchyma cells have begun to bulge into their lumina. The commencement of the process of occlusion of the earliest elements may clearly be recognized at 1 cm. above the insertion of the leaf. The metaxylem is still immature and does not function, but the younger protoxylem has matured to take the place of the disrupted earlier elements. At a still higher level the metaxylem begins to stain, and one or two protoxylem elements still conduct (fig. 15). The lacunae left by the disruption of the protoxylem continue to stain for some time after their occlusion by parenchyma cells has begun, and apparently play some part in conduction, although they soon become filled with a mucilaginous substance which stains deeply with Delafield's haematoxylin. Finally the lumina of the protoxylem elements are completely occluded by the inward growth of the neighboring parenchyma, and the metaxylem alone is functional. The isolated rings or elongated spiral coils are visible closely imbedded in the parenchyma (fig. 16). One large cell may more than cover the site of the vanished lumen. A longitudinal section shows that the smallest rings are very widely separated, and the distance between the rings or turns of a spiral decreases as the element to which they belonged lay nearer the metaxylem (was of later origin). The level at which the metaxylem begins to conduct depends upon the age of the leaf. In a leaf just appearing above the pseudostem the metaxylem did not stain at all, although the solution rose through the entire length of the sheath in the protoxylem; in another, which had spread its lamina but was still elongating, the metaxylem stained at 3 cm. above the insertion of the sheath.

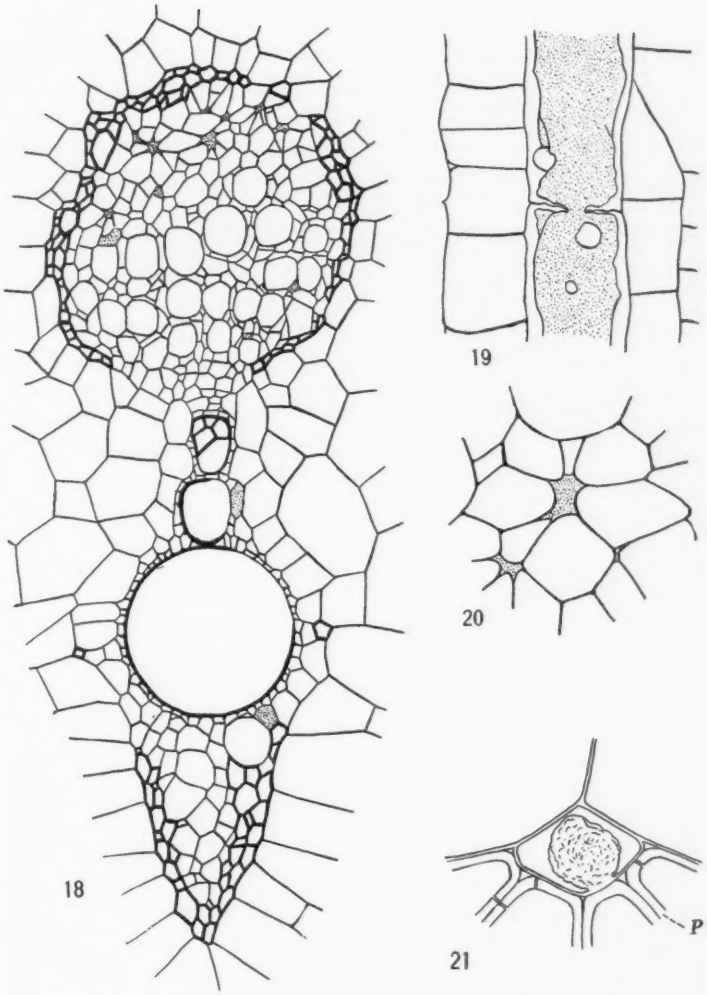
The sequence of events just outlined agrees essentially with that described for *Musa* by Miss BUCHHOLZ (**2**). *Musa* belongs in the third of the four classes of monocotyledons which she distinguishes on the basis of the fate of the protoxylem in organs with intercalary growth. If we trace the course of the ascending water current, we

find that it begins to rise in the innermost and earliest elements; as it passes upward it moves outward to younger and younger elements, following their wider lumina in place of the narrower ones which are becoming occluded. Finally the stream enters the large metaxylem tracheids, in which it continues up into the lamina.

In mature sheaths it is possible to cut sections passing between the widely separated rings which show no trace of the presence of the protoxylem, so completely is the scar healed (figs. 16, 18). The single large tracheid is very wide, reaching 0.25 mm. in the diameter of its lumen. According to HABERLANDT (9) the tracheids of *Musa* reach 1 cm. in length. The smaller bundles in the septa, and the inner and outer walls, do not exhibit a sequence of protoxylem elements, and are not functional in the intercalary growing zone. The bands of thickening of both protoxylem and metaxylem are suberized, but only occasionally does the metaxylem give a faint lignin reaction.

The cross-sectional area of the phloem is considerably greater than that of the persisting xylem. The sieve tubes (fig. 18) are large and conspicuous, and arranged with some regularity. The larger are 65 μ in diameter and 2.4 mm. long. The sieve plates are transverse or very slightly oblique. The lateral walls of sieve tubes, companion cells, and cambiform cells are penetrated by small circular pits. In immature bundles the walls of the sieve tubes are very noticeably thickened, but they become thinner as the phloem matures. In the more peripheral portions of the bundle small schizogenous slime passages are formed. These are surrounded by 4–7 cells which bulge into the intercellular cavity, and are filled with a mucilage which stains deeply (fig. 20).

The xylem and phloem of the larger bundles are each partially surrounded by a separate horseshoe-shaped sheath of mechanical tissue. The gap between the two rings is always opposite the commissural connectives, and through this break in the sheath the commissural bundles enter the longitudinal bundles (fig. 18). The thickness of the sheath depends upon the position of the bundle. In the large bundles in the longitudinal septa it is 1–3 cells thick. The relative development of prosenchyma increases in proportion as the bundle lies closer to the outer surface of the sheath. At the same time



FIGS. 18–21.—Fig. 18, vascular bundle from mature sheath; ×100. Fig. 19, immature mucilage vessel from petiole, showing perforation of end wall between two of cells composing vessel; ×345. Fig. 20, schizogenous mucilage passage from phloem of petiole; ×325. Fig. 21, stegma from bundle in sheath; *P*, prosenchyma; ×675.

the amount of conducting tissue is diminished, and the outermost bundles are mere strands of thick-walled fibers, without vascular tissue, which are largely responsible for the rigidity of the pseudostem. The sheath around the large interior bundles of the septa, especially its xylem portion, is often of rather thin-walled cells, and does not extend around the large tracheid. This is true especially at the base of the leaf sheath. Fig. 18 shows a sheath of moderately thin-walled cells incomplete around the large tracheid. The thinner fibrous sheaths are interrupted by gaps in which the cells are thin-walled, and serve as transfusion cells.

The composition of the walls of the fibers varies considerably in different leaves, and even in different portions of the same leaf sheath. The strands of fibers alone and the thick sheaths of greatly thickened cells in the outer wall of the leaf sheath are usually lignified. The thinner sheaths around the large inner bundles often give a distinct lignin reaction with phloroglucin, but in other places are suberized and not lignified.

FIG. 22.—Portion of tracheid from commissural bundle of sheath.

Accompanying the mechanical sheath on its outer face are groups of cells containing concretions of silica, the so-called stegmata. The stegmata are small cells with unthickened walls; they are short with blunt ends, and arranged in longitudinal rows. Each contains a roughly spherical mass of silica, apparently devoid of any organic basis (9), which almost completely fills the lumen, and is attached to the wall of the cell by a short stalk (fig. 21).[7]

Surrounding the bundles, outside the mechanical sheath, is a jacket of starch-filled parenchyma cells. This starch sheath persists even where it is separated from the vascular tissue by a sheath of 4–6 layers of heavily lignified fibers. The orientation of the principal bundles of the sheath, petiole, midrib, and lamina is always normal, the xylem toward the adaxial, the phloem toward the abaxial side.

[7] They may best be observed by following a modification of the method described by KÜSTER (11). Hand sections are dehydrated in alcohol and then mounted for observation in benzol, which gives the concretions a faint red tint. The cell walls may be stained in safranin, etc., while in the alcohol.

The curvature of a septum in the midrib may sometimes cause an easily detected physical reversal of morphologically normal orientation. In the longitudinal septa of sheath and midrib the axis of some of the smaller bundles without protoxylem is sometimes turned sideways, and occasionally the orientation is completely reversed.

The longitudinal bundles are connected at all levels by numerous commissural bundles, which lie in the plane of the horizontal dissepiments. However, only extremely rarely do these commissures strike across the dissepiment; normally their course is around its margin, where it joins the longitudinal walls of the lacuna. Here the commissural bundle may extend around two or three sides of the lacuna, sending out arms which connect with the small tracheids (commissural connectives) of the longitudinal bundles near its course. Other commissures occur in the outer wall. The commissural bundles consist of a small amount of phloem, and usually a single narrow tracheid, the thickening of which is reticulate and often of very intricate pattern (fig. 22).

11. Mucilage ducts and cells

External to the bundles but parallel to them are numerous mucilage ducts. These arise by the end-to-end union of a longitudinal series of parenchymatous cells, and correspond to the latex vessels of Haberlandt (9). Often several mucilage-bearing cells lie end-to-end, apparently without connection between them; at other times a large central pore penetrates the end walls separating the cells, and in some cases this wall almost completely disappears. A stage in the fusion of two mucilage cells to form a vessel in a young petiole is represented in fig. 19. Isolated cells having contents giving the same staining reactions as those of the mucilage ducts occur throughout the leaf, including the vascular bundles, and especially the phloem, where the cambiform cells may contain mucilage (fig. 18). In the lamina, sieve tubes which themselves contained a similar substance were discovered. The schizogenous passages in the phloem in all regions contain the same substance, and probably have a similar significance to the plant (fig. 20). The mucilage ducts are the *Milchsaft-Gefässe* of Wittmack. According to this author, their contents, in *M. ensete*, are rich in potassium chloride, potassium

oxalate, and tannins. The sheath of the Gros Michel is also rich in tannins.

12. Aerenchyma and air chambers

The lacunae arise by the tearing apart and destruction of the tissues of certain regions in the interior of the sheath; they are rhexigenetic in the sense of De Bary. A cross-section through a young sheath near its base shows regions of comparatively large cells, which have begun to round off, separated by smaller, closely packed cells. The former are in the lacunar region, the latter will become the longitunal septa. A longitudinal section through the lacunar region at a slightly higher level reveals transverse layers of intact cells separated by thicker layers of large, thin-walled cells devoid of much contents. The former give rise to the transverse septa, the latter are being pulled apart by the elongation of the sheath to form the lacunae. The disrupted tissue is evidently resorbed, since the mature lacunae are quite free from cell fragments. The dissepiments are of two kinds. Both contain the characteristic aerenchyma (fig. 6), made up of a reticulum of stellate cells. Morren (**14**) describes and figures the formation of the stellate cells. The septa of the first type are formed by 1–4 horizontal layers of this stellate aerenchyma alone. Those of the second type are composed of a single or several layers of small parenchyma cells situated between an upper and a lower stratum of the stellate cells, each of which may be one or two cells in thickness. The thick septa are more common in the sheath than in the midrib. Starch grains are numerous in both types of cells, and according to Greve (**8**) the transverse septa, particularly the central layer of parenchyma of the compound type, are a principal tissue for the storage of starch. The lacunae are usually filled with air, but in wet weather may become partially or almost completely filled with water, a circumstance noticed also by Baccarini (**1**, fide Greve).

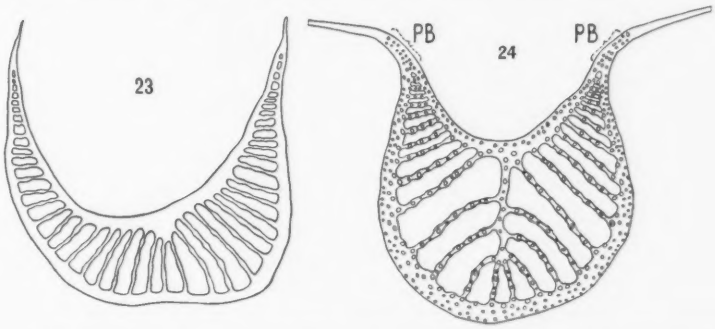
Lying free on the surface of the transverse septa are numerous large raphid cells filled with bundles of acicular crystals of calcium oxalate. These cells are furnished with processes resembling those of the star-shaped aerenchyma, and by these they are attached to the latter. They may attain the very great size of 322 μ in length and 81 μ in diameter. Raphid bundles are numerous in giant cells

imbedded in the tissues of all portions of the leaf, and isolated monoclinic crystals of calcium oxalate are abundant in the parenchyma everywhere.

B. PETIOLE AND MIDRIB

13. Gross structure

The petiole and midrib resemble each other in general structure so strongly that they may be described together, occasional differences being noted where necessary. At the top of the pseudostem each sheath begins to become narrower and thicker. The central



Figs. 23, 24.—Fig. 23, cross-section at about middle of petiole, showing transition from structure of sheath to that of midrib; ×0.5. Fig. 24, cross-section of midrib, near base of lamina of large leaf, position of principal vascular bundles indicated by circles; *PB*, pulvinar band; ×1.

lacunae contract toward the adaxial surface; they are crowded inward by those lateral to them, and these finally meet in the central line, overarching the central lacunae and pushing them away from the adaxial wall (fig. 23, and cf. figs. 17 and 24). This change from the typical structure of the sheath to that of the midrib occurs gradually in the proximal portion of the petiole, and the typical cross-section of the midrib is presented by its distal portion. This structure will be sufficiently clear from fig. 24. It is only necessary to add that the midrib, which near its base is very massive in large leaves, 4.5 cm. from side to side and 2.7 cm. from top to bottom along the median line, tapers gradually toward the apex, the lacunae becoming fewer and smaller. It does not, as in many monocotyledons

(for example, the related *Strelitzia reginae*), blend into the blade before the apex of the leaf is reached, but is distinct throughout the length of the latter (fig. 12).

14. Peripheral tissues

As in the sheath, the peripheral tissues on the two sides of the petiole and midrib differ markedly. Considering first the lower surface, the epidermal cells are of the same general type as those of the sheath, but shorter. A thick external wall of cellulose is covered

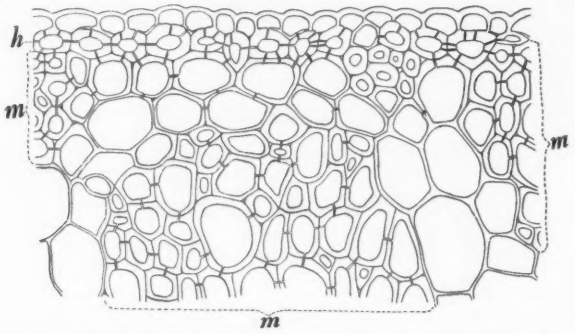


FIG. 25.—Peripheral region of lower side of midrib; *H*, lignified hypodermal layer; *M*, fibrous strands; ×350.

by a thin cuticle, which bears a very heavy waxy "bloom," similar to that of the lamina mentioned later. The hypodermal layer is made up of cells rectangular in surface view, their long axes coinciding with the length of the midrib. These cells are very thick-walled and lignified, and their walls are penetrated by prominent radial pits. Beneath this is a second layer of cells with walls slightly thinner, but also lignified and pitted. Within these are 2–3 layers with walls slightly thickened, and suberized rather than lignified (fig. 25). The lignification of the walls of the hypodermal cells becomes incomplete, and that of the second layer disappears near the distal extremity of the midrib. The continuity of all strata interior to the hypodermal layer is broken by numerous strands of lignified prosenchyma. These fibrous bundles are very abundant in this region, the outermost abutting on the hypodermal layer itself. They are of all

sizes, ranging from 5–6 to several dozens of cells in cross-section, and they lie so close together that usually not more than three cells of the ground tissue intervene between two adjacent strands (fig. 25).

The upper surface bears an epidermis covered with a moderately thick cuticle, reaching 2 μ in thickness. Beneath this are 2–3 layers of large colorless cells, elongated from side to side of the midrib, forming a hypodermal water tissue. Their general appearance in surface view resembles that of the corresponding cells of the lamina, reproduced in fig. 32. Except at the edges of the midrib, near the pulvinar bands, the walls of these cells are slightly thickened and suberized, but not lignified as in the case of the lower surface. The fibrous strands are not nearly so numerous beneath the upper as beneath the lower epidermis.

15. Internal anatomy

The distribution of the vascular bundles between the walls and the longitudinal septa resembles that of the sheath, and is shown diagrammatically in fig. 24. The general character of the bundles is much the same as in the sheath, and the reaction of the cell walls of corresponding elements is similar. The larger bundles show the same succession of occluded protoxylem elements, but the process of occlusion was not studied here. As hinted previously, the fibrous strands in both outer and inner walls, as well as in the septa, are more numerous than in the sheath. These strands are usually but not always lignified, but if not lignified at least they are suberized. The commissural bundles are of the same type as in the sheath, but more often pass directly across the transverse septum from side to side of the lacuna, instead of hugging the longitudinal walls. When the petiole and lower portion of the midrib are split in half longitudinally, and one side only placed in a solution of eosin, the solution readily crosses over in the commissural tracheids, and moves downward into the midrib and blade of the other half below the upper end of the cut (50 cm. below in one experiment which lasted 3 hr. 20 min.). The transverse septa are of the same type as in the sheath, but the thinner kind, composed of aerenchyma alone, without the small-celled central parenchyma, are relatively more numerous here.

It may be profitable to consider for a moment the mechanical features of the midrib and petiole, which must support in an almost horizontal position an enormous expanse of lamina. The lower surface, the term extended to include all of that portion beneath the pulvinar bands, is surrounded by a woody shell at least two cell layers thick, strengthened on the inner side by numerous longitudinal ribs, the fibrous strands. This woody shell with its supports, because of the curvature of its walls, is well qualified to resist buckling under the compressional stresses to which it is subjected. The prosenchymatous strands, both surrounding the vascular tissue and distinct from it, concentrated in the upper wings of the midrib and petiole, are adapted to support the tension to which their location exposes them, and are more efficacious here than an equal number would be if collected in the central portion of the upper wall, since in their actual position they are farther removed from the lower side.

16. Pulvinar bands

The diurnal movements of the lamina were described by Johow (**10**) and later by Trelease (**20**). In the early morning, or during a wet day, the two halves of the lamina stand out almost in a horizontal plane on either side of the midrib (fig. 27). During a bright day the lamina halves bend downward, bringing their lower surfaces together beneath the midrib (fig. 26). Where the lamina has been torn into strips, the individual segments move together synchronously, almost as though the lamina were intact. This movement occurs rapidly during the morning of a dry day, and undoubtedly is very effective in decreasing transpiration, since most of the stomata are on the lower surface, and the profile position assumed greatly diminishes the amount of radiant energy absorbed. The change in the appearance of the plant is very striking. The movement is not a passive drooping brought about by the wilting of the leaves, but is produced by the activity of the pulvinar bands, which bend downward along the sides of the midrib in response to changes in the turgor of their tissues.

The pulvinar bands lie along the edges of the midrib, flanking it for its entire length, and merge on the external side into the blade (fig. 24 *PB*). They differ from the tissues on either side of them in

that they contain no lacunae. Their thickness is between 1.2 and 1.8 mm. The ground tissue is of rounded parenchyma cells, resembling the motor tissue of the pulvini of dicotyledonous leaves. Beneath the epidermis is a water tissue composed of 4–6 layers of clear

FIG. 26.—Banana plant photographed at noon on a bright day; "false pinnae" hanging downward in profile position.

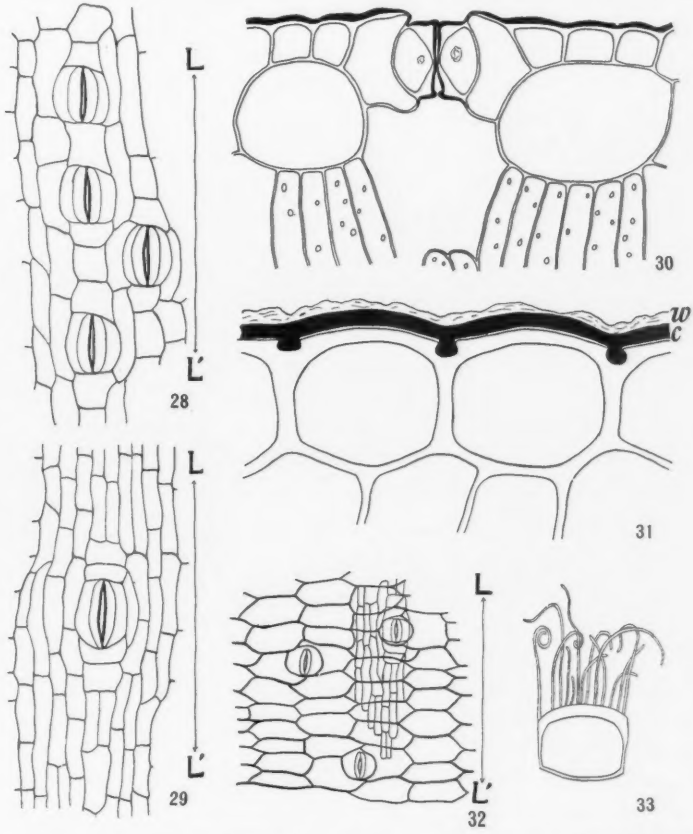
cells. Next below this is a chlorophyllous layer of close set, rounded (not palisade) cells. This borders below a region of thin-walled, rounded parenchyma cells, containing little chlorophyll, which occupies the central portion of the organ. In this tissue are situated the vascular bundles, which here are surrounded by a very thick mechanical sheath, probably an adaptation to prevent injury to the

vascular elements by the bending of the pulvinus. The small metaxylem elements (commissural connectives) are much more numerous in this region than elsewhere in the leaf, and several of the large tracheids may occur in the cross-section of each bundle. Anasto-

FIG. 27.—Same plant as fig. 26, just after sunrise following morning; "false pinnae" standing out almost horizontally.

moses between the bundles are frequent. Below the central parenchyma are 2–3 layers of large, upright prismatic cells, in which run a few fibrous strands. Abutting on the lower epidermis is another water tissue of 2–3 layers of clear cells, slightly thick-walled, elongated in a direction parallel to the midrib. The exact mechanism of the movements of the organ and the functions of the various tissues have not been determined.

A peculiarity of the cuticle over the upper epidermis deserves mention. Like that over the rest of the upper surface of the lamina, this cuticle is rather thick. Distinct knobs project from its inner



FIGS. 28–33.—Fig. 28, lower epidermis of lamina in surface view (line *LL'* drawn parallel to veins); ×315. Fig. 29, upper epidermis; ×315. Fig. 30, transverse section through stoma and accessory cells of upper epidermis, cuticle indicated by heavy black line; ×570. Fig. 31, upper epidermis from pulvinar band of mature leaf; *C*, cuticle; *W*, waxy layer; from a section stained with Grübler's cyanin and aqueous safranin; ×1100. Fig. 32, upper hypodermal layer, as seen from inner surface, showing also some of cells of upper epidermis; ×135. Fig. 33, rods of wax forming the bloom, from lower epidermis of leaf just expanded; ×about 800.

surface where it overlies the anticlinal walls of the epidermal cells, and penetrate the latter, the cellulose wall, which is here rather thick, being hollowed out to receive them (fig. 31). Often the knob is distinctly constricted at the neck, and the epidermal wall narrows around the constriction. The cuticle is thus anchored to the epidermis by a ball-and-socket device. In portions of the cuticle isolated by treatment with concentrated sulphuric acid to destroy the cellulose, the knobs appear as a series of distinct, equidistant projections which outline the epidermal cells. Often there appears to be a narrow gap between the epidermis and the cuticle over the middle of an epidermal cell. The cuticular knobs are confined to the upper surface of the pulvinar bands and the portion of the midrib immediately adjoining. In other regions the cuticle comes down in points over the anticlinal walls of the epidermal cells, but is not knobbed. The arrangement appears to be associated with the motility of the pulvinar band, and perhaps aids in securing the cuticle to a surface which undergoes great changes in curvature.

C. LAMINA

17. External characters

The outline of the lamina is ovate-oblong. The apex is blunt, but the two sides are unlike (fig. 12). The left half, which is external in vernation, extends farther down the petiole than the right (fig. 9), and in large leaves this difference may amount to 12 cm. Wittmack states that in *M. ensete* the covered half extends farther down the petiole than the one which is external in vernation, and is likewise markedly broader (external half 41 cm., internal 50 cm.). In many species of the Marantaceae, Musaceae, and Araceae, it seems to be a general rule that the half of the lamina which is covered in vernation is broader than the exterior half, often (as in *Calathea* spp., *Musa cavendishii*, etc.) pronouncedly so. The general rule holds in the case of the Gros Michel, but the difference between the halves is not so great. In the few leaves of greenhouse plants available, the right side was 2–10 per cent broader than the left.[8]

[8] The writer is indebted to P. R. White for measurements of leaves growing at the New York Botanic Gardens. He neglected to observe this point during his sojourn in Jamaica.

The thickness of the blade increases gradually from margin to midrib. The difference is quite considerable, and a large leaf may be 3.5 times as thick next to the midrib as at the margin. Both the

TABLE II

INTERNAL TOPOGRAPHY OF A LAMINA 340 CM. LONG

POSITION		MEASUREMENTS			
Distance above base (cm.)	Distance from pulvinar band (cm.)	Thickness of lamina (μ)	Depth, upper epidermis + water tissue (μ)	Depth of palisade tissue (μ)	Depth of lacunae (μ)
10	0	718	68–91	76–95	.....
	0.6	568	76	91	317
	1.2	518	65	95	267
	2.5	484	61	99	251
	5.0	434	61	95	217
	10.0	351	57	87	134
	16.0(M)*	267	57	76	57
120	0.0	935	76	122	601
	1.9	752	57–76	118	484
	3.8	685	53	118	418
	7.5	618	49	114	351
	15.0	501	53	110	267
	30.0	367	38–53	95	156
	44.0(M)	267	42	84	57
220	0.0	768	65	122	484
	1.6	618	49	122	367
	3.3	585	53	114	317
	6.5	534	49	110	284
	13.0	451	46	106	234
	26.0	334	27–46	95	134
	39.0(M)	251	38–57	84	57
330	0.0	534	57	106	251
	0.6	468	57	95	217
	1.2	434	57	95	200
	2.3	401	53	95	184
	4.5	384	30–53	95	167
	9.0	317	30–53	91	134
	14.0(M)	301	53	80	80

* M = margin.

absolute and proportional increase in thickness are greater in the middle region of the leaf than at the base or apex. The rate of increase in thickness is not uniform from margin to midrib, but becomes greater as the midrib is approached (table II). As is apparent from the table, the increase in thickness is accounted for largely

by the increase in depth of the lacunar region, the palisade and dermal regions changing relatively little.

From the strong midrib the pinnately arranged veins follow an S-shaped course to the margin. All of the veins of the same region of the leaf are very nearly parallel. The branching of the bundles and the outward curvature of the S occur principally in the pulvinar band, and the forward curvature does not assume prominence until the vein has approached to within 5 mm. of the margin, so that across the breadth of the blade the veins follow a course which is practically straight, except at the extreme base and apex of the lamina. At their distal extremities the veins bend forward and unite in the marginal bundle, which practically surrounds the lamina. The apparent parallelism of the veins is not exact. At the base of the lamina they are directed backward as they leave the pulvinar band, forming an angle of 100°–120° with the midrib. At the apex their course is forward, the angle becoming 55°–77°, while the middle veins are nearly perpendicular to the midrib. As the result of this, all of the veins diverge slightly, and any strip across the blade, such as that torn by the wind, is wider at the margin than at the midrib.

Two orders of veins are apparent upon the most superficial examination. The strong or principal veins lie at the apex of an upward curvature of the lamina, and accordingly are situated above its general surface. This fold gives the lamina a ribbed appearance (fig. 12), which is in nowise caused by the vein itself, for this is not appreciably thicker than the immediately adjoining portions of the blade. The weak or subordinate veins lie flat in the plane of the blade. The anatomical differences accompanying these superficial distinctions are discussed later. Both the strong and weak veins exhibit great differences in development, and there are gradations between the two. Alternating with the strongest veins there is usually a somewhat weaker vein of the same character, easily distinguished from the subordinate veins lying between them. WITTMACK distinguishes six orders of veins in *M. ensete*, and presents a diagram showing their derivation from a single bundle. Since the number of subordinate veins between each pair of principal veins is not constant, it is probable that in the Gros Michel there is no very definite scheme of ordination.

Practically all of the veins which extend into the blade from the midrib continue until they reach the marginal bundle, or stop within 1.5 cm. of the margin. However, new subordinate veins may arise blindly between any pre-existing pair of them in any region of the blade, and they have been observed to begin less than 1 cm. from the margin. The intercalated veins do not arise by the branching of the original veins; their connection with the vascular bundles of the midrib is by means of the commissural bundles linking them with adjacent veins. The length of the intercalated veins may be less than 1 cm., in which case the vein ends blindly as it began, but the majority run out into the marginal bundle. As a result of this intercalation of veins, there are more subordinate veins between each pair of principal ones at a few centimeters in from the margin than near the midrib. The increase in the number of veins by intercalation is greatest at the base of the lamina, where the average increment in the two leaves for which counts were made was 36 per cent. At the middle of the leaf the increment was 20 per cent and at the apex 9 per cent. Because of the intercalation of veins, all regions of the lamina are equally well supplied with vascular bundles. The average distance between subordinate veins is practically the same at the margin as at the midrib, the variation in average separation amounting at most to 0.03 mm. The interval between veins is very nearly the same in all regions of the lamina, the averages lying between 0.19 and 0.26 mm. The average distance between the strongest principal veins is about 1.3 cm., but great variation is found here. Usually between 35 and 60 subordinate veins, as well as the weaker principal vein, occur between each pair of the strongest veins, the count being made at the midrib. The number may rise to 100, however, in large leaves. Taking 0.24 mm. as the average interval between veins, a lamina 394 cm. long would have about 17,000 veins on each side near the midrib, and correspondingly more near the margin.

One other feature in the external topography of the lamina seems worthy of notice. On many leaves one can distinguish longitudinal streaks running along the lamina parallel to the midrib, often giving a faint suggestion of ribs (faintly visible in figs. 12 and 13). The ribbed appearance is due to nothing more than kinks in the blade, slight furrows being impressed upon the upper surface, with

corresponding low narrow ridges upon the lower, although occasionally the direction of the curvature is reversed. The furrows are conspicuous chiefly in consequence of their great length, stretching from end to end of the lamina. These furrows are associated with no structural differentiation of the blade, but result from unequally distributed pressure upon the young leaf still rolled within the pseudostem, causing folds which are not entirely lost by the lamina in expanding. The number and distribution of the furrows on the two sides of the lamina lend weight to this view. Generally there are 14–20 on the right half at its widest part, and 4–5 on the left, although it is difficult to give figures, because some furrows are so indistinct that one is at a loss to decide whether they should be considered as such or not, and there is great variation between individual leaves. The spacing also differs on the two halves. On the right half the furrows are very close together near the margin, and the interval between successive furrows increases as the midrib is approached. On the left half the 4–5 furrows are almost equidistant. These two circumstances agree with the number and diameter of the coils in vernation (see Section 7). The most distinct furrows are near the right margin of the leaf, corresponding to the narrower coils in this region.

In vernation the coiled lamina usually does not show two perfect helices in cross-section, but, especially in the later stages, the inner helix is generally flattened in the plane of the expanded lamina (fig. 8). In some cases the coils may be flattened like cloth rolled around a board, and even folded back upon themselves in the form of a V. This departure from the ideal form in vernation is responsible for the permanent disfigurement of the tissues of the right half of the lamina. The furrows on the left half probably result from pressure exerted on the blade where it crosses the keel of the midrib in vernation, this being rather sharp at certain stages.

18. Dermal system of lamina; number of stomata

Both the upper and lower epidermis are composed of straight-walled cells, almost rectangular in surface view, elongated in a direction parallel to the veins (figs. 28, 29). The upper epidermis is covered by a rather thick (1–2 μ) cuticle, while that over the lower

is considerably thinner (0.5 μ or less). The lower surface of the lamina is covered by a thick "bloom" which gives it a glaucous color. In leaves just expanded it may be seen that the "bloom" is formed by innumerable close-set rods of wax, which are very slender and often curl up at the free ends (fig. 33). The rods soon crumple and slump together, and in old leaves they are represented only by irregular waxy masses. The "bloom" on the young leaves is so thick that it can be rubbed off on the fingers in large quantities as a white powder.

The stomata are all oriented with their long axes parallel to the veins and to the length of the epidermal cells. On the lower surface, where they are most numerous, they lie over the lacunae in narrow bands stretching across the blade from midrib to margin, while the adjacent strips of epidermis over the veins are almost devoid of them. The stomata of the upper epidermis are somewhat larger than those of the lower, the guard cells of the former measuring about 32 μ in length, those of the latter 28 μ, and other dimensions are in proportion (cf. figs. 28 and 29). The ventral ridges are not very pronounced, and the front and back cavities of the pore are small or indistinct. The cutinization of the guard cells is indicated in fig. 30. The passage between the guard cells is lined with a cuticle which extends over the inner periclinal walls, and over the outer walls is continuous with the general cuticle of the epidermis. The guard cells are bordered by accessory cells (figs. 28, 29, 30), and the short broad cells at either end of the stomatal group have a characteristic shape. The thick outer walls of the accessory cells are constricted to a more or less pronounced hinge where they join the guard cells. The statement of SCHUMANN (**20**) that the rods of wax which make the "bloom" on the lower epidermis bend over the stomata to form *einem oben offenen Dom* seems to the writer to convey an exaggerated idea of the definiteness of the structure in question and the part played by it in reducing transpiration. Individual rods arising from neighboring cells do bend over into the free space above the guard cells, but, in the Gros Michel at least, are arranged to form no definite structure.

The average of 35 separate determinations of the number of stomata per sq. mm. of the lower surface of the lamina is 168.5.

The variability in different leaves, and different regions of the same lamina, is so great that the figure just given has little significance. Individual counts (of an area of about 0.7 $\overline{mm.}^2$) varied from a maximum rate of 261 per $\overline{mm.}^2$ to a minimum of 96 per $\overline{mm.}^2$ Certain definite tendencies of distribution were manifest in all of the

TABLE III

DENSITY OF STOMATA ON LAMINA

REGION	NUMBER OF STOMATA PER SQ. MM.					
	Lower surface*			Upper surface†		
	Midrib	Middle	Margin	Midrib	Middle	Margin
Base	134	169	143	36	31	26
	Mean for base = 149			Mean for base = 31		
One-fourth up	223	229	128	69	55	34
	Mean = 193			Mean = 53		
Half-way up	184	214	114	87	62	39
	Mean = 181			Mean = 63		
Three-quarters up	232	242	224	78	66	44
	Mean = 233			Mean = 63		
Apex	259	261	182	50	54	56
	Mean = 234			Mean = 41		
Mean	206	223	158	64	54	40

* Lamina 391 cm. long, 104 cm. in greatest width. Stomata in an area of epidermis 0.686 $\overline{mm.}^2$ counted in each case.

† Lamina 353 cm. long, 97 cm. in greatest width. Stomata in an area of 1.372 $\overline{mm.}^2$ counted in each case.

leaves of which a series of counts was made. Thus the basal quarter of the lamina has fewer stomata per unit area than those portions distal to it (table IV).

TABLE IV

Average no. of stomata at base of lamina . . . . . 124.1 per $mm.^2$ (maximum 169, minimum 96)
Average no. of stomata in central half of lamina . . . . . 174.8 per $\overline{mm.}^2$ (maximum 240, minimum 97)
Average no. of stomata at apex of lamina . . . . . 170.6 per $\overline{mm.}^2$ (maximum 261, minimum 117)
Ratio, central half 1.41 : apex 1.37 : base 1.00

In every leaf the average density in the central and apical portions was greater than the maximum density found at the base of the same leaf. Likewise there are more stomata in the region of each blade near the midrib than in that near the margin, except at the base of the lamina, where the order seems reversed (table V).

TABLE V

Average no. of stomata near midrib ..... 185.2 per $\overline{mm}.^2$ (maximum 259, minimum 145)
Average no. of stomata near margin ..... 148.9 per $\overline{mm}.^2$ (maximum 224, minimum 97)
(Counts at base of lamina not included in average)
Ratio, midrib 1.24:margin 1.00

The maxima and minima overlap because some leaves have everywhere more stomata than others. When the counts at the margin and the midrib of a given level of the same lamina are compared, the midrib exceeds the margin in every case except at the base. There are some indications that the density of stomata midway between midrib and margin is greater than in either the interior or marginal region of the blade (table III). The individual counts of a representative leaf are recorded in this table.

On the upper surface the average based upon 28 counts is 40.1 stomata per sq. mm. The maximum density found was 89 per $\overline{mm}.^2$, the minimum 3 per $\overline{mm}.^2$ Here again the basal portion fell behind the more apical regions in number of stomata. The ratios of frequency were central half 1.83:apex 1.75:base 1.00. The stomata are again more numerous in the central than in the marginal region of the lamina, and the difference is even more pronounced than in the lower surface, the ratio being midrib 1.51:margin 1.00. Finally, it may be recorded that large leaves near the crown of the plant seem to have more stomata per unit of area (both surfaces) than smaller leaves from lower down.

A larger number of determinations might have been made, but since the tendencies of distribution just recorded repeated themselves on every leaf with much constancy, these conclusions are stated with a fair degree of confidence, although the figures given are of value only as examples of what differences are to be expected. There would be little point in presenting those obtained in greater detail. Per-

haps their principal worth is to serve as a caution to those who might attempt to employ large leaves, such as that of the banana, in experiments in which the rate of transpiration or assimilation is to be correlated with the number and condition of the stomata. WITTMACK'S figures on the number of stomata of the leaf of *Musa ensete*, 260 per $\overline{mm}.^2$ on the lower surface, 7 per $\overline{mm}.^2$ on the upper surface, are given here for comparison with those of the Gros Michel.

Beneath both the upper and lower epidermis there occurs a water tissue of thin-walled cells with clear contents (figs. 30, 35, 36, etc.). The cells in this tissue lie with their long axes parallel to the midrib and transverse to the veins; they are crossed with the epidermal cells (fig. 32). The thickness of the water tissue varies according to the region of the lamina. The upper water tissue is usually two layers thick just within the marginal bundle, somewhat farther in it is often single-layered, but usually double, near the midrib it becomes triple, and still closer it is often quadruple. The transition from the double to the triple condition is illustrated in fig. 36. The tissue is usually thicker where it overlies a vein than in immediately adjacent regions. The lower water tissue is often double near the margin, and double or triple near the midrib; over most of the lamina it is single or sparingly double.

Where one of the stronger of the longitudinal ribs just described crosses one of the principal veins, a node or swelling, paler in color than the rest of the lamina, may often be seen upon the upper surface of the leaf. Since a node at these places is not of constant occurrence, and they are absent from many ribbed leaves, the swelling is obviously not the cause of the rib, but rather a result of its presence. It is caused by a hypertrophy of the cells of the water tissue at the points in question, which elongate enormously in a direction normal to the surface of the lamina. Where the water tissue is two-layered only the cells of the inner layer elongate, and where it is three-layered only the inner two, the outermost layer remaining normal and being pushed passively upward. Not only the cells above the principal vein, but those on either side, often passing over several subordinate veins, exhibit the hypertrophy (fig. 34). Cells of this character may reach 317 μ in depth, about thirteen times the normal dimension, and a three-layered water tissue, of which the two inner

layers have hypertrophied, may become 568 μ thick. The hypertrophy at the intersection of vein and rib occurs only in the case of furrows upon the upper surface; where the upper surface is ridged, the swelling is manifest along that portion of the vein between the ridges. Hypertrophy of the water tissue over a principal vein may occur close to the midrib at points where it is not associated with a longitudinal furrow.

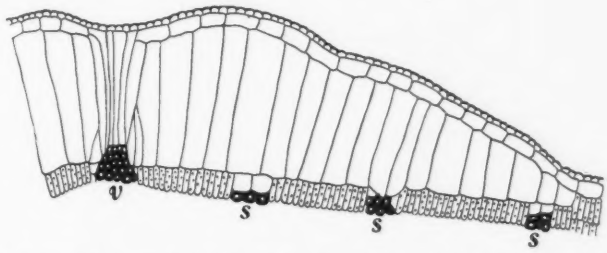
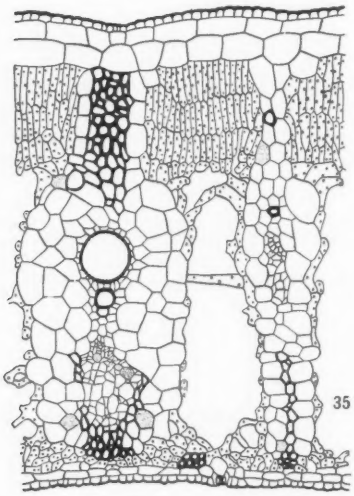


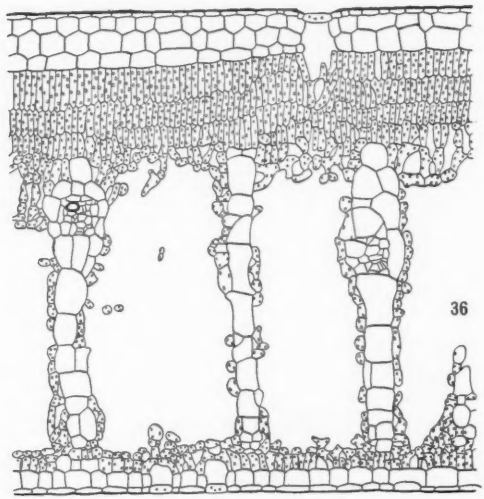
FIG. 34.—Hypertrophied cells of hypodermal layer at intersection of principal vein (*V*) with longitudinal furrow; *S*, subordinate vein; ×60.

18. INTERNAL TISSUES OF LAMINA

The interior of the lamina is divided by the veins into a series of canals which stretch from midrib to margin (fig. 35). Each canal is cut up by transverse septa into chambers or lacunae (fig. 36). The average length of the lacunae (the dimension parallel to the veins) increases markedly from margin to midrib. The average distance between transverse septa at the base of the lamina was 95 μ at the margin, 182 μ midway between margin and midrib, and 230 μ near the midrib. In the middle of the lamina the corresponding figures were 68–122–204 μ; near the apex 75–119–127 μ. On the contrary, the average width of the lacunae is greatest near the margin, where the veins are thinnest, and diminishes toward the midrib; it ranges between 170 μ and 73 μ. (For the depth see table II.)

Between the veins and above the lacunae lies the palisade tissue (figs. 35, 36), composed of three layers of distinct palisade cells, with frequent suggestions of a fourth. The palisade tissue is not interrupted by the transverse septa, and forms a continuous narrow band from midrib to margin. It is somewhat deeper near the





FIGS. 35, 36.—Fig. 35, section of lamina transverse to veins, at about midway between margin and midrib; both veins of "subordinate" type; ×135. Fig. 36, section parallel to veins, septa to right and left being supplied with vascular bundles, while central septum is devoid of them; ×135.

midrib than at the margin, but its thickness does not increase nearly so rapidly as that of the lamina as a whole (table II). The lacunae are lined with spongy parenchyma, which occurs beneath the palisade and above the ventral water tissue, and borders the veins and transverse septa. Frequent elongated chlorophyllous cells, somewhat resembling the algiform cells of moss capsules, stretch across the lacunae.

The vascular bundles are surrounded by one to several layers of clear parenchyma cells, elongated parallel to them. The larger bundles contain a single large tracheid strengthened by twelve or less spiral bands, according to the size, and several smaller tracheids (commissural connectives) below them (fig. 35). The spiral bands of the xylem are suberized. The weakest bundles contain only a few very narrow tracheids; more rarely, near the margin, no xylem at all. The phloem is always present and more fully developed. The latter contains the same schizogenous mucilage cavities described for the sheath, and sieve tubes with the same dark-staining contents occur. Mucilage ducts are present in the parenchyma surrounding the bundles, and individual mucilage-filled cells occur in the phloem.

Accompanying all but the weakest veins are two strands of prosenchyma, one above and the other beneath the vascular bundle. The fibers in the lower strand are lignified, but those of the upper are suberized. The lower strand is accordingly more durable, and in portions of a leaf along a tear it persists after the upper strand has decayed. In the weakest veins, the upper strand is reduced to one or two isolated fibers in cross-section. In strong veins the lower strand extends out as arms which protect the phloem, but in weak veins this portion of the prosenchyma is reduced to a horizontal plate of cells one layer thick (as in the vein to the right in fig. 35). Strands of mechanical cells sometimes occur beneath the lacunae, apart from the veins, especially near the midrib, and at times are associated with a wall which projects upward into the lacuna, but does not reach the palisade tissue.

The principal veins, lying at the apices of the transverse folds of the lamina, are distinguished from the subordinate ones by a much greater development of the two mechanical strands accompanying them, and by the series of protoxylem elements which are dis-

rupted and occluded by neighboring parenchyma cells. The subordinate veins do not contain protoxylem. The stronger of the subordinate veins contain as much metaxylem and phloem as the principal veins in the same part of the leaf.

Except where a commissural bundle is present, the transverse septa are a single layer of cells in thickness, with a broken layer of spongy mesophyll on either side. The central cells have clear contents, and in surface view are very irregular in shape. The irregular projections and re-entrant angles interlock and a close tissue is formed. The mesophyll cells are elongated parallel to the wall, much branched, and very complex in outline. One, two, or three together of the central cells (in septa where there are no commissures) may become thick-walled mechanical cells. These cells are often very broad and long, stretching most of the way across the septum, and have the same irregular outline as the other cells of the central layer. The walls are penetrated by prominent pits. These mechanical elements are not numerous, but are most frequent near the margin of the lamina.

The commissural bundles which connect the veins run through the transverse septa. They consist of a single tracheid (in cross-section) and a small amount of phloem, but no mechanical elements, and resemble the commissures in the sheath and midrib (fig. 36). Their course is usually not horizontal, because the bundles of the veins which they join are not always at the same level. By no means every septum contains a vascular bundle, and as many as fourteen septa devoid of a bundle may intervene between two having them, although often only a single one intervenes. If the veins supplying a certain portion of the lamina are severed near the midrib, so that its supply of water must travel longitudinally through the commissural tracheids, and the petiole is then placed in solution of eosin, the commissural bundles are stained and stand out clearly when held up to the light. They appear as thin, tortuous red lines with frequent anastomoses, the general direction of which is parallel to the midrib. They are not distributed at random among the septa, but arranged as though with some regard to their continuity as water courses transverse to the veins. The commissures are often continuous across one or more veins; however, usually the septa equipped with com-

missures are not in line on the two sides of the vein, but one is displaced slightly in or out with reference to the other, so that the sap must move along the vein for less than the length of one lacuna to pass from one commissure to the other. Thus they do not form an independent system like the veins, and their continuity as a water course is dependent upon the latter. The apparent anastomoses occur where two commissures join a vein almost opposite a single one on the other side. The efficiency of the commissural bundles as water courses was demonstrated in many experiments, a few of which are recorded here.

1. A strip of the lamina extending 35 cm. in the direction parallel to the midrib, and about 15 cm. in the direction of the veins, was cut from a large leaf while the dew was still upon it. The severed ends of the veins were smeared with vaseline. The lower end was cut into fringes to increase the absorbing surface, and the strip (*A*) was hung with this end dipping into a 0.75 per cent solution of eosin. Another strip (*B*) from the same leaf was cut with the length perpendicular to the midrib and parallel to the veins, and hung in the same manner beside the first. The preparations were cut under water. The solution could ascend the strip *B* through the veins, but in *A* it could move upward only through the commissural bundles. The strips were left thus for four hours on a sunny morning (August 27, 1926). At the end of that time the solution had stained *A* 20 cm. above the lower end, while in *B* it had risen 34 cm., to within 1 cm. of the top, and probably would have gone farther had the strip been longer.

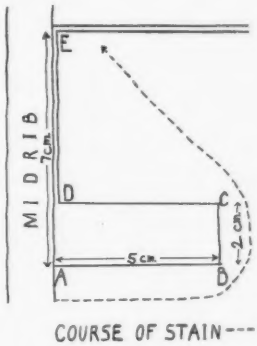


FIG. 37.—Diagram to illustrate Experiment 2.

2. A lamina was cut as shown in fig. 37. To reach the portion marked *E* it was necessary for the solution to travel 5 cm. outward along the vein from *A* to *B*, then 2 cm. upward through the commissural bundles from *B* to *C*, then inward through the veins and upward through the commissures from *C* to *E;* altogether at least 7 cm. upward through the commissures. The severed petiole was

placed in a 0.75 per cent solution of eosin at 9:00 A.M., and the experiment terminated at 10:00 A.M., August 25. The stain had reached *D*, and the upper margin of the segment 1.5 cm. out from *E*. *A* was about 150 cm. above the cut end of the petiole.

3. The lamina was torn across from margin to midrib, and then severed from the midrib for a distance of 34 cm. upward, so that a loose flap hung downward, attached to the midrib only by the commissural bundles. The cut petiole was placed in a 0.75 per cent solution of eosin. After 2 hours 26 minutes the stain had reached 25 cm. below the last intact vein at the top of the flap, having moved this distance through the commissural tracheids, in addition to 220 cm. upward through petiole and midrib.

20. Margin

The young blade as it emerges from the pseudostem is margined by a delicate wing, white or tinged with red, but containing no chlorophyll, about 2.5 mm. broad. About 170 μ thick where it joins the marginal bundle, the wing narrows to a fine edge, and the outermost portion is scarious and transparent. It is composed entirely of thin-walled parenchyma, with an upper epidermis of small unthickened cells, and on the lower surface are individual cells containing mucilage, similar to those occurring in the epidermis of the precursory appendage. The marginal wing withers almost as soon as the leaf is exposed to the air, and dies off as far as the marginal vascular bundle.

Within the wing there occur a few small tracheids, followed by a very large tracheid about 125 μ in diameter, which occupies most of the cross-section of the lamina at this point, and a few somewhat smaller ones inside it. These elements must be regarded as forming a marginal bundle which is a separate entity, and not merely the longitudinal continuation of the veins, since the latter contain as they approach the margin no tracheids of this size. An almost continuous band, 1–1.5 mm. broad, of much smaller tracheids stretches inward from the large tracheids, and represents the marginal continuation of the veins. Very little phloem is present. The first mechanical tissue strengthening the margin occurs as narrow bands, 1–2 cells deep, of not very thick-walled fibers lying above and below

the bundles interior to the large marginal tracheids. Within this, strands of mechanical cells are present at intervals, and represent the distal extremities of the fibrous strands of the veins.

21. Splitting of lamina

The newly expanded leaf is entire. Older leaves are torn by the wind into narrow strips, parallel to the veins, which are usually distinct from one another as far as the pulvinar bands, and the tear may even proceed longitudinally along the midrib for a short distance (fig. 38). The leaf then appears to be pinnately compound, and the strips into which it is torn may well be called "false pinnae," since their physiological significance is the same as that of pinnae (compare the banana leaf in fig. 38 with the coconut leaves in the upper left corner of that figure).

The line of the tear made by the wind follows no course predetermined by the structure of the lamina, other than that it must run parallel to the veins; there are no anatomical differences which cause it to lie between one pair of veins rather than another. On the other hand, the resistances offered to the initiation or continuation of the tear are almost negligible. The marginal wing is short lived and is supplied with no mechanical elements; the marginal bundle contains a few weak mechanical strands which eventually bend parallel to the veins, and so present but a slight obstacle to the tear; the transverse septa include only isolated, inefficient thick-walled cells, and the commissural bundles are equipped with no mechanical elements to retard or stop its progress. Lippitsch (12) has made an interesting study of the development in several genera of the Scitamineae of those mechanical features which may retard the transverse tearing of the lamina. Many of these are better protected than *Musa sapientum*, and are still incapable of maintaining their integrity in the face of the wind. He believed the splitting to be autogenous, in that "primary tears" at right angles to the margin and penetrating the outer portion of the marginal bundle are initiated by the tension set up by the drying and contraction of the wing. These tears are continued inward as "secondary tears" by the action of external agents, of which the wind is the principal. Considering the ease with which the lamina may be split inward at any point, it

does not seem necessary to postulate any mechanism for the initiation of the tear to account for the laceration of the enormous lamina under the force of the breeze. According to PETERSEN (16) the cleft often begins in the body of the lamina and thence proceeds inward and outward. One certainly sees many incompleted splits

FIG. 38.—Old leaf, much frayed by wind, no longer able to support itself; such effete leaves wither in this position and drape the pseudostem.

of this character, but apparently they also are to be attributed to the shearing force of the wind. KARSTEN (quoted by GOEBEL 7) found that the margin of the lamina of *Heliconia dasyantha* dies before the central protion has ceased to enlarge. The dead margin persisting, tensions arise which result in the splitting of the lamina under the force of impact of rain drops, etc. GOEBEL (7) traces a suggestive

series in the compounding of monocotyledonous leaves. In the banana the false pinnae arise from the action of external forces on the mature leaf. In *Cyclanthus bipartitus* the time of the splitting of the two-lobed leaf is pushed forward, and occurs during its unfolding, resulting from tensions already present. In the palms the compounding of the leaf occurs still earlier, in the bud, where certain strips of tissue die or become slimy, isolating from each other the strips which make the pinnae.

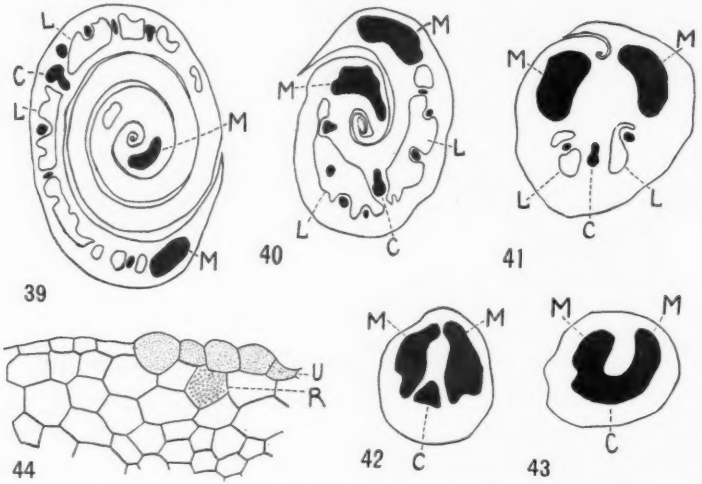
In the Gros Michel the behavior of the tissues adjacent to the tear is similar to that reported for *Musa ensete* by LIPPITSCH. The tissues die off as far as the second or third vein from the line of separation, and the pinna is bordered by a narrow (0.2–0.4 mm.) band of brown dead cells. The parenchyma surrounding this vein, especially on the side toward the wound, the water tissue, and the epidermal cells become thick-walled and suberized, the outer anticlinal walls often becoming considerably thickened. Often, however, the palisade cells between the first and second veins become suberized, at times in 5–6 vertical rows, as well as the water tissue and epidermis in continuity with them. At any rate, a complete wall of suberized tissue, often very irregular, closes off the wound. Fungal infections, however, sometimes spread inward from the tear as large yellowing areas. The wound left by the dying off of the margin is also closed off by suberized cells, so that the false pinna is protected on three sides by suberized membranes. Wound cork is never formed. LIPPITSCH points out that the tissue lost as a result of splitting represents a smaller outlay of material than would be necessary adequately to strengthen the leaf. He believes that this habit therefore conforms to the principle of conservation of material; and the lack of mechanical protection, inviting as it does the splitting, may be regarded as adaptive.

D. PRECURSORY APPENDAGE

22. STRUCTURE AND POSSIBLE FUNCTION

The cylinder formed by the convolute leaf narrows at its apex into the precursory appendage (figs. 9, 11), which in large leaves may reach 15 cm. in length. The coiled halves of the lamina continue upward as part of the appendage, which in its basal portion is

a helix in cross-section (figs. 39, 40). They gradually become narrower, and finally merge into the terete structure which is characteristic of the upper portion of the appendage (fig. 42). The veins at the apex of the lamina bend inward and upward very sharply, changing their course through as much as 135°, and joining the marginal bundle, with which they continue upward into the appendage. Because the appendage is so intimately connected with the blades



FIGS. 39–44.—Figs. 39–43, transverse sections of precursory appendage at various levels; ×12. Fig. 39, at base of appendage; fig. 40, 1 cm. above base; fig. 41, about 2.5 cm. above base; fig. 42, about 6 cm. above base; fig. 43, 7–8 cm. above base; *C*, central vascular bundle; *M*, marginal bundle; *L*, lacuna. Fig. 44, peripheral region of appendage in transverse section; *U*, mucilage cells; *R*, raphid bundle; ×135.

it must be torn off, along with the end of the right half, before the lamina can uncoil.

The appendage is a direct continuation of the midrib, and the bundles and lacunae of the latter extend upward into the base of the former (fig. 39 *C*, *L*). The lacunae contract and finally drop out, and the smaller bundles fuse and disappear. At about one-third the length of the appendage above the base only three bundles remain, the central one representing the continuation of the bundles of the midrib (fig. 42 *C*), and the other two the marginal bundles of the lamina (fig. 42 *M*). Already at the base of the appendage the mar-

ginal bundles were the most prominent, and as they continue upward their cross-sectional area relative to that of the entire appendage increases, until they occupy a large proportion of the cross-section of the organ. Finally the three bundles fuse and become indistinguishable. This was observed to happen in two ways. The marginal bundles may remain distinct from each other and fuse with the central bundle at their inner edges, forming an open ring (fig. 43), or they may first fuse with each other by their outer edges, and later with the central bundle at their inner edges, forming a closed ring. The single bundle present in the upper quarter of the organ becomes solid. The large marginal bundles are composed almost entirely of fairly wide tracheids, with a small amount of phloem scattered among them, and a larger portion of it at the inner edge of each bundle. In the predominance of xylem over phloem, these bundles resemble the marginal bundles of the lamina. Mechanical elements are absent from the appendage.

The ground tissue of the appendage is a parenchyma composed of thin-walled cells only slightly longer than broad. Cells containing raphid bundles are very frequent. The epidermis contains numerous stomata with large substomatal chambers, and large cells, elongated in the direction of the long axis of the appendage, which contain a deeply staining mucilage, and project above the general surface of the organ. These occur as isolated cells or are continuous in large groups (fig. 44). In addition, there are present epidermal cells of the usual type. The appendage never contains chlorophyll, and is white so long as it is still tightly inclosed in the pseudostem. When it emerges from the top of the latter it has already begun to turn brown, as a result of the withering and discoloration of the epidermal cells. Long before the lamina has completely emerged the appendage is black and shriveled, although it usually remains attached until it is torn off by the expansion of the lamina. The end curls up in drying, but the organ never assumes the corkscrew form described for other species.

Whatever may be the physiological function of the appendage, its mechanical significance seems clear.[9] It serves to maintain the

[9] For an interesting view on the morphological nature of the appendage, which space does not allow me to discuss here, see A. ARBER, On the leaf tips of certain monocotyledons. Linn. Soc. Jour. Bot. **45**: 467–476, and the literature there cited.

young leaf in its coiled condition, and to prevent its fouling against the sheath which surrounds it during its long progress upward through the pseudostem. If the lamina ended abruptly, the folding back of its ends through friction against the sheath and the consequent entanglement of the leaf would be possible. By its tapering form the appendage is well adapted to push its way through the close-packed organs of the pseudostem, and to make a passage for the lamina which follows. This passage may be further aided by the lubricous surface created by the mucilaginous epidermal cells. The conclusion of GOEBEL (**6**) that the appendage is an *Abschlusskörper* seems a sound one.

The physiological function, if any, of the appendage in the Musaceae is not so clear. RACIBORSKI (**17**), whose studies were devoted principally to lianas, found that the stomata, vessels, intercellular spaces, and chlorophyll apparatus of the appendage mature earlier than in the body of the leaf, and that it transpires and assimilates before the latter. He observed that the earlier maturation of tissue is also true in the banana, although here photosynthesis is out of the question. Lianas, with their retarded leaf development, present conditions peculiar to themselves, and quite foreign to the banana. GENTER (**5**) suggested that the appendage is of service in closing off the central cavity of the pseudostem and preventing the access of rainwater and foreign matter to the young leaf. He also observed that if the appendage is freed from the surrounding sheaths and placed in a moist atmosphere, it guttates through the stomata. However, there is no experimental evidence that the organ has any non-mechanical physiological value to the plant.

In conclusion, it may be well to point again to the large number of scars which the leaf of the banana receives during the usual course of its development, both from the dying off of temporary organs and the imperfect adjustment between its parts. The withering of the margin and the precursory appendage, the tearing off of the end of the right side, all leave extensive scars which surround the lamina. These changes would occur in the most sheltered greenhouse as well as in the field, and perhaps it is not stretching the point too far to include the withered margins of the false pinnae in the same category, since the leaf is not adjusted to its habitat until

it has become falsely pinnate, and probably no large leaf on the plantation escapes being torn. If we turn to the internal tissues, the complete destruction of the protoxylem and the rhexigenetic origin of the lacunae should be included. The duration and fate of temporary organs during organogeny and development have received more attention from zoologists than from botanists, probably because plants are not so strongly individualized as animals. But the leaf of the banana is a highly individualized unit, which shows in a striking way the phenomenon of the abscission of temporary parts.

The investigations reported in the present article were made entirely on plants of the Gros Michel variety growing at Platfield, a plantation of the United Fruit Company near Richmond, Parish of St. Mary, Jamaica, where the writer spent a portion of the summer of 1926 as a guest of the Company. The work was completed in the botanical laboratory of the Johns Hopkins University. The writer desires to express his indebtedness to Dr. JOHN R. JOHNSTON, Director of Agricultural Research, for suggesting these investigations and to him and the other officials of the United Fruit Company, for making it possible to carry them out; to Professor DUNCAN S. JOHNSON, Johns Hopkins University, for many helpful suggestions, and to the superintendent at Platfield, Mr. T. D. KIEFFER, and Mrs. KIEFFER, for the innumerable kindnesses which made his sojourn on the plantation enjoyable as well as scientifically profitable.

Summary

1. The aerial shoot of the banana is leafy almost to the top of the pseudostem, and bears the largest leaves produced by the plant.

2. The phyllotaxy changes with the age of the plant, from under 2/5 to 4/9.

3. The lateral buds are not axillary, but arise *opposite* the axils.

4. The unrolling of the convolute leaf is accomplished only after the tearing away of a portion of the lamina.

5. Stomata occur on all the inclosed portions of the sheaths and stem.

6. The hypodermal layer of the outer surface of the sheath be-

comes first suberized, then lignified, as the sheath is pushed toward the exterior of the pseudostem.

7. The protoxylem elements of sheath, midrib, and principal veins are disrupted and their lumina occluded by parenchyma cells.

8. The lower surface of midrib and petiole is strengthened by two hypodermal strata of lignified cells.

9. A peculiar mode of anchoring the cuticle over the pulvinar bands is described.

10. Longitudinal furrows in the lamina are caused by pressure in the bud.

11. There is considerable variation in the density of stomata in different portions of the same surface of the lamina.

12. There is a marked increase in the number of veins near the margin over that near the midrib.

13. The efficiency of the commissural bundles in water transport was clearly demonstrated by experiment.

14. The wounds caused by the splitting of the lamina, and the dying off of the margin, are closed by the suberization of pre-existing cells.

15. The marginal bundles of the lamina become the most important vascular supply of the precursory appendage.

ARLINGTON, MARYLAND

[*Accepted for publication May 31, 1927*]

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CYTOLOGICAL STUDIES IN THE GENUS TYPHA[1]

MURIEL V. ROSCOE

(WITH PLATES XI, XII AND FOURTEEN FIGURES)

In a report on the flora of the Boston district (13), there is appended to the notation of the presence of *Typha latifolia* L. and *T. angustifolia* L. the following: "Where the two species grow together there are all kinds of intermediate forms. This is especially noticeable in the big swamps at West Cambridge."

This tendency to develop intermediate forms has been found to be by no means limited to West Cambridge, but is general in localities where the two species grow in any proximity to each other; thus forms grading into the typical *latifolia* and *angustifolia* are common. Further, it is known that hybrids of these two species occur in northern Europe.

This investigation was undertaken to ascertain the chromosomal conditions in the parent species and the suspected hybrids. Also, before the close of the investigation, some material from New Zealand, *T. angustifolia* L. var. *muelleri* Graeb. was received. Since *T. latifolia* is absent from New Zealand, the material was examined to note any resemblances and differences between this New Zealand variety and the *T. angustifolia* of New England.

Materials and methods

Material of both *T. angustifolia* and *T. latifolia*, as well as of forms lying systematically somewhere between the two, was obtained growing in various localities in Massachusetts. *T. latifolia* was also gathered in various places in Nova Scotia; while I am indebted to Professor JEFFREY for material of *T. angustifolia* var. *muelleri* from New Zealand.

Chromo-acetic (0.75 per cent) solution was used as a fixative except in the case of the New Zealand material, which was preserved in Carnoy's fluid. After the anthers were cut with a sharp razor, the

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

material was placed immediately in the fixative and pumped. Collecting was confined to the warmer parts of fine, warm days.

Following imbedding in nitrocellulose, the buds were cut at thicknesses of 5 and 10 μ, and stained with Haidenhain's iron-alum haematoxylin. For the study of division stages no counter-stain was employed, while for pollen study safranin was used as a counter-stain after haematoxylin. The sections were studied with the assistance of a 1.5 mm. Zeiss apochromatic objective and a no. 12 compensating ocular.

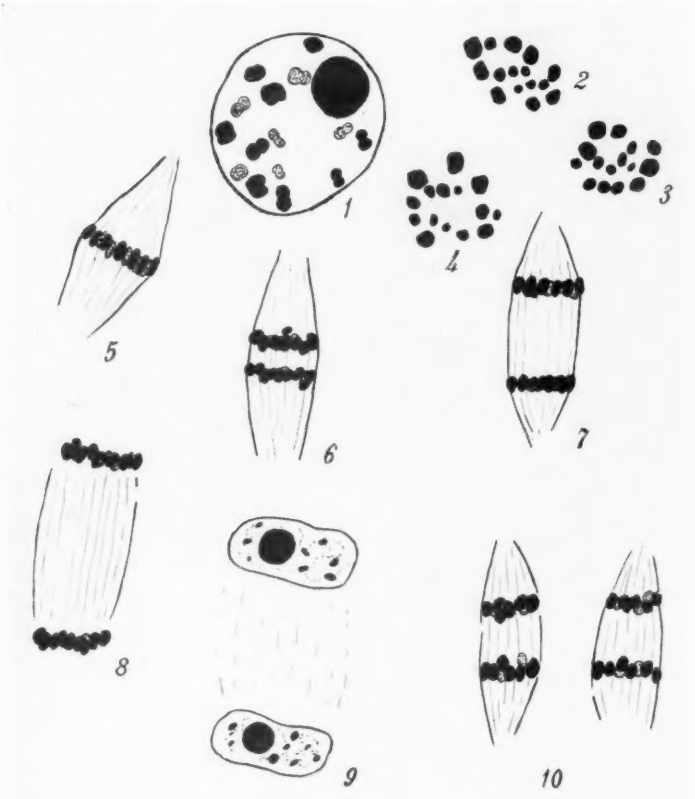
Observations

1. T. LATIFOLIA.—The divisions of *T. latifolia* are illustrated in figs. 1–10, and were contained in material collected from one pond margin in Nova Scotia.

The difficulties presented by diakinesis were great, and were due not only to the presence of a large nucleolus combined with the small size of the chromatin elements, but also to the intimate union of the latter in bivalent formation. In the most favorable cases, the situation is as shown in fig. 1, where all of the chromatin units seem double, and diakinesis may be settled as revealing fifteen bivalent chromosomes, which vary considerably in size. It is generally true that three of the bivalents in diakinesis are larger than the others (fig. 1), and a variation in the size of the remainder can be noted. Polar views of metaphase plates are definite in establishing the chromosome number as fifteen, and also show that the different sizes suggested by diakinesis are actually existent. It has not, however, been considered possible to arrange the chromosomes in any absolute categories, for the number of large and of small elements varies. Withal, the size difference of the chromosomes is dependent to some degree upon their plane of orientation on the spindle. The variations in size are thus most conspicuous in polar view of metaphase, and are usually not discernible in profile views of the plates or in subsequent stages. Also some preparations show a greater size difference than others, suggesting that the relative size is not an invariable quantity.

There is nothing extraordinary featuring the general course of meiosis, and heterotypic division (figs. 2–9) is completed with the formation of the two daughter nuclei, which are organized for the

resting period previous to the second division. In this, as in the first division, nothing abnormal or irregular is noted. A representative



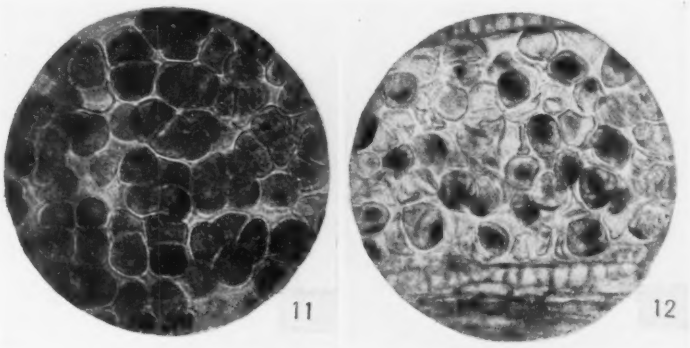
FIGS. 1–10.—*T. latifolia:* fig. 1, diakinesis; figs. 2–4, heterotypic metaphase, polar view; fig. 5, heterotypic metaphase, profile view; figs. 6–8, heterotypic anaphase; fig. 9, interkinesis; fig. 10, homotypic anaphase; ×approximately 2600.

anaphase is shown in fig. 10. At the conclusion of the homotypic, the normal tetrad of four similar microspores is developed.

It is significant that the pollen which is produced after such divisions is morphologically perfect. All the grains reach maturity,

and, when shed, retain the peculiar tetrad formation which is characteristic of *T. latifolia* (fig. 11). A study of pollen of this species when growing removed from *T. angustifolia* has shown it in all cases to consist of perfect grains.

In the establishment of these conclusions, some hundreds of pollen mother cells in division have passed under observation, and all stages submitted to repeated verification; thus cells showing metaphase plates, polar view, have been counted in upward of a hundred cells.



FIGS. 11, 12.—Pollen: fig. 11, *T. latifolia;* fig. 12, *T. angustifolia;* ×approximately 600

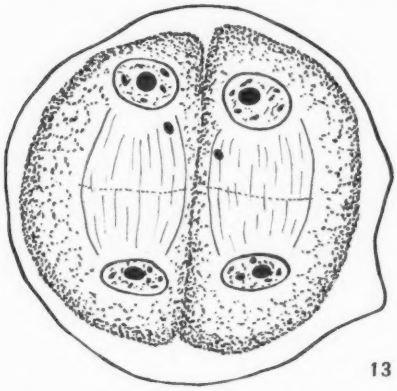
It may further be noted that some proportion of sterile pollen is frequently found in material gathered from what appears to be "pure" *T. latifolia* growing in New England, but such a pollen condition has never been found in any *latifolia* pollen from plants isolated as they are in Nova Scotia.

2. T. ANGUSTIFOLIA HYBRID.—This form approached *T. angustifolia* in appearance, but had a somewhat broader leaf and shorter interval than is usually found in the typical species. In such plants prophase usually discloses fifteen units. Metaphases may be regular and show only bivalents, but frequently metaphases with both bivalents and univalents are found. In such cases it is possible that a very loose union exists and some of the homologous chromosomes are early dissociated. Anaphases both regular and irregular have been observed, but all the chromosomes become included at inter-

kinesis. At the close of the homotypic division, however, some of the chromosomes are occasionally left out of the daughter nuclei (fig. 13).

A somewhat unusual pollen mother cell is shown in fig. 14, where three nuclei of as many sizes are present, and contain varying numbers of chromatin elements.

3. T. ANGUSTIFOLIA.—Plate XI shows meiotic divisions for *T. angustifolia*. Fig. 15 represents an early prophase, in which some chromatin-like material is in actual passage from one cell to another.



FIGS. 13, 14.—*T. angustifolia* hybrid: fig. 13, homotypic telophase; ×approximately 2600; fig. 14, abnormal pollen mother cell with three nuclei; ×approximately 700.

This material seems to be intimately connected with the chromatin masses within the nuclei, one of which is becoming involved in the transmission. A number of cases showing black-staining material of this sort have been seen in *Typha;* such phenomena are conspicuous, but from the lack of a detailed study, no attempt is made to explain them.

Diakinesis is variable in regard to the number of chromosomes which appear. The same difficulty experienced in understanding this phase for *T. latifolia* is met here, but is enhanced in this case by the dissimilarity of different nuclei. In the one illustrated (fig. 16), eighteen elements may be counted, of which it is believed that all except the very smallest are bivalents; that is, we have twelve bivalents and six univalents. However, as many as twenty-two units

have been counted in other nuclei. This fluctuation in number may be explained as due to: (1) the disharmony of the parental chromosomes resulting in loose union; or (2) the discrepancy in numbers of the parental chromosomes. It is not yet possible to decide which of the two factors is in operation. The difficulty is increased because no chance to check by metaphase plates is presented, on account of the lagging of chromosomes on the spindle.

The manner in which the bivalents and univalents conduct themselves after the formation of the spindle varies from the course noted for *T. latifolia*. Figs. 23 and 24 are representative of early metaphase, and it will at once be noted that the departure from the norm is extreme. Not only is there delay in coming to the plate, but the variation in size, form, and relation of the chromosomes is great. Fig. 24 thus shows bivalents (both large and small), apparent univalents, and two cases where univalents have aligned themselves to appear as elongated masses of chromatin. Fig. 23 is even more anomalous, and shows sixteen chromosomes, of which one lying in the plate region is very large, and approached in size only by one of the two at the upper pole. The situation is pronounced. The suggestion might be made that these are two persistent nucleoli, but such does not seem to be a valid explanation, inasmuch as more than one nucleolus has never been observed in prophases, and also the prophase nucleolus is much larger (fig. 16). It is possible, if not probable, that the two masses are spherical aggregations of chromatin.

Later metaphases (figs. 19, 20) show that a larger number of the chromosomes reach the plate, but that always some univalents are left on the spindle. Fig. 20 is a rather rare condition, for usually more laggards appear than do in this figure. Consequently counts of polar view of such a stage are valueless. A large number of observations have shown fifteen chromosomes in such a "pseudo-plate" (fig. 17), but in two cases sixteen were realized, and one cannot be assured that the number is not still higher. From the fact of inconstancy in number in diakinesis, coupled with the variation in chromosome size, and the small size of all the elements concerned, as well as from the extreme irregularity of position assumed by the chromosomes on the spindle, it has been found impossible to settle upon a definite numerical value for the form under consideration.

Anaphase (figs. 18, 21) is usually irregular. Interkinesis frequently shows small masses of chromatin left outside the daughter nuclei, which may or may not form micro-nuclei.

One much elongated spindle with a peculiar disposition of the chromosomes was conspicuous (fig. 22). The spherical mass at the lower pole is comparable with similar masses noted in fig. 23, and is probably an abnormality resulting from the heterozygous character of the parent species.

A few homotypic divisions passed under review, and these showed some lagging, which, however, was not as pronounced as in the heterotypic. The end result is the formation of pollen which is crushed and sterile (fig. 12).

4. T. ANGUSTIFOLIA VAR. MUELLERI.—Plate XII shows stages in the division of pollen mother cells of material collected in New Zealand, and identified as *T. angustifolia* L. var. *muelleri* Graeb. by Dr. LEONARD COCKAYNE of Wellington, New Zealand.

Figs. 25–28 illustrate the heterotypic division, the figures of which are distinguished by regularity of conduct of the chromosomes. A feature of interest is presented by the number of chromosomes, which is thirty. This New Zealand variety of *T. angustifolia* is therefore a tetraploid form. Fig. 28 shows the thirty chromosomes at metaphase. There is a vast difference in the present instance between the general size of the elements, as contrasted with those in New England species of *Typha*, and it is apparent that the chromosomes are much smaller in the New Zealand variety. The homotypic division is likewise regular in the distribution of the chromosomes (fig. 29).

Discussion

1. CHROMOSOME NUMBERS.—The haploid chromosome number of fifteen, while not of any significance in itself, is a number seldom recorded for the higher plants.

2. NORMAL MEIOSIS.—Where meiosis is normal and is followed by the production of only fertile pollen, we have the expected condition for pure species. *Typha latifolia*, on the basis of these two features, together with its diploid chromosome number, is undoubtedly of uncontaminated origin.

3. ABNORMAL MEIOSIS.—Where meiosis is abnormal, and is followed by varying percentages of sterile pollen, the situation is linked

up with hybridity. This conclusion is warranted in view of the outstanding cytological contributions of recent years, which were inaugurated by ROSENBERG'S studies (15–17) on *Drosera*.

Usually irregularity of division is due to differences, either numerical or qualitative, in chromosome inheritance. Thus irregularities resulting from the hybridization of parents containing different numbers of chromosomes is seen in *Drosera obovata* ($2\times=30$), whose parents, *D. rotundifolia* and *D. longifolia*, contributed ten and twenty chromosomes respectively (17). COLLINS and MANN (8), however, decided from their researches on *Crepis* hybrids that chromosome dissimilarity and not chromosome number determines abnormal reduction. WINGE (19) expresses similar ideas when he says: "it is therefore certain that only such gametes as harmonize physiologically—or better, perhaps physiogenetically—can enter into the formation of a duality such as the sporophytic organism." He later concludes: "On the whole, imperfect reduction is associated with the hybrid nature." One thus feels justified in interpreting constantly occurring meiotic abnormalities as indicative of hybridism.

Viewed in such light, slight irregularities in *Typha* result when the parents are of nearly equivalent constitution, whereas greater abnormalities accrue when the parents are genetically dissimilar. The difference in the number of chromosomes noted in diakinesis and spindle figures of *T. angustifolia* may be due either to the difference in the numbers or to the physiological dissimilarity of the chromosomes contributed by the parents. In the latter case, the failure to form bivalents would increase the number of chromatin elements and explain the numbers recorded. At least, unlikeness of chromosomal units (either qualitative or numerical) leads to irregular divisions, and hence to the formation of gametes which either fail to reach maturity or else contribute an entirely different chromosome equipment to a new generation. In the latter case, unless an auto-regulative device is inaugurated whereby the chromosome number is reduced, these irregularities will be perpetuated.

4. TETRAPLOIDY.—The appearance of thirty haploid chromosomes in the New Zealand variety *muelleri* of *T. angustifolia* was a striking repetition of phenomena reported for many genera of plants. BLAKESLEE, BELLING, and FARNHAM (4) have limited the usage of

the term tetraploidy to the doubling of homologous chromosomes in a somatic cell, but the customary use of the term is to denote the condition of any species containing twice as many chromosomes as a closely related form. Since it is difficult to know whether or not chromosomes are homologous, if these elements are very small and manifest no recognizable morphological differences, the more general definition of the term seems preferable.

Perhaps the most outstanding case of tetraploidy in which the history is known is that described by Miss DIGBY (**11**) for *Primula kewensis*. The first appearance of this species was in 1899, when one aberrant individual appeared, obviously as a result of a natural cross between *P. floribunda* ($\times = 9$) and *P. verticillata* ($\times = 9$). In 1900 the same hybrid was obtained by artificially crossing the two supposed parents. The hybrid proved sterile, although, like its parents, it possessed the diploid number of chromosomes ($2\times = 18$), and all the flowers were thrum-eyed. In 1905 one pin-eyed individual appeared, and, by fertilizing it with pollen from a thrum-eyed individual, a seedling was developed which contained eighteen haploid chromosomes and was fertile. *P. kewensis* is an instance of a tetraploid form originating in material known to be of hybrid constitution.

BREMER (**5, 6, 7**) has made an extensive study of *Saccharum*, including many species and species hybrids within the genus. He relates a haploid number of fifty-six for *S. spontaneum* and of forty for many different races of *S. officinarum*. He finds further that Kassoercane, which is probably a spontaneous hybrid of *S. officinarum* and *S. spontaneum*, has sixty-eight chromosomes, and concerning the origin of such a number, BREMER (**6**) relates the following:

> The diploid chromosome number of these hybrids, which approximately reaches 136, can be explained only by assuming that, when fertilisation with a male nucleus of *S. spontaneum* (glagah) occurs, the number of chromosomes in the egg cell of the sugar-cane (*S. officinarum*) doubles, so that not 40 but 80 sugar-cane chromosomes meet with 56 *glagah* chromosomes, with which they form the diploid number 136. After reduction, the haploid chromosome number 68 then results.

In a recent *Rosa* contribution, BLACKBURN and HARRISON (**2**) find *R. wilsoni* falls in line with Miss DIGBY'S *Primula kewensis* and

BREMER'S *Saccharum*, both of which followed hybridization. *R. wilsoni* has a somatic number of forty-two, and undoubtedly was derived from a cross between *R. pimpinellifolia* (balanced tetraploid) and "some *Tomentosa microgene*" (unbalanced pentaploids, whose microspores when functional carry seven chromosomes and the egg cells twenty-eight). "With *pimpinellifolia* as seed parent, the cross should have 14+7 (=21) in its somatic nuclei." Since *R. wilsoni* has a chromosome number of forty-two, "it has, therefore, like *Primula kewensis*, doubled its original complement. In doing so, again like that hybrid, it has attained fertility."

Species with twice as many chromosomes as related ones are reported in many genera: *Dahlia* (**12**), *Aegilops* (**1**), *Rosa* (**18**), and *Rubus* (**14**). DENHAM (**9, 10**) counted the chromosomes in thirty-two varieties of cotton, and found New World and Egyptian cottons were tetraploid ($\times = 26$) and Asiatic cottons diploid ($\times = 13$). No explanation is offered by him concerning the appearance of the larger number. The numbers given by WINGE for *Atriplex patulum* and for *Chenopodium bonus-henricus* illustrate chromosome doubling in the Chenopodiaceae. The list of such cases might be extended to cover most of the genera which contain large numbers of species, for tetraploid forms are common within polymorphic groups.

From a consideration of the known results of other workers, and from his own extensive observations, WINGE says the doubling of the chromosome number should be explained "not as a cleavage of the chromosomes, but as an indication of hybridization, and thus an addition of the chromosomes." Such a result is due to the coming together of gametes of too little harmony. BLACKBURN and HARRISON (**3**) find in the Salicaceae normally acting tetraploid species, both in *Salix* and *Populus*. They view hybridity as a spur to the duplication of chromosomes, and suggest that in general tetraploid species probably arose in one of two ways: (1) two nuclei become included in one cell; (2) the chromosomes become fragmented without subsequent cell division, an hypothesis advocated by WINGE. A third method seems justifiable where diakinesis reveals only unpaired chromosomes, due to complete disharmony of the parental gametes, a conclusion which follows from WINGE'S ideas.

There is a certain similarity between the chromosomal condition

of the previous cases and of *Typha angustifolia* var. *muelleri*. In plants like *Primula kewensis*, where the ancestry and cytological conditions are known, it is easier to postulate the origin of a doubled number, or at least to note that, regardless of the method of doubling, the tetraploid condition is connected with heterozygous ancestry. In the *Typha* under discussion, nothing is known of the parent stock, either historically or cytologically; but in comparison with *T. latifolia* of Eastern America, it is a form which has doubled its chromosome complement. It distributes its chromosomes equally in sets of thirty, and forms tetrads normally. The percentage of viable pollen, in the absence of mature grains, has not to date been estimated.

By analogy with the development of tetraploidy within the genera *Primula*, *Saccharum*, and *Rosa* in known hybrid stock, the possibility is offered that the chromosome number in *Typha angustifolia* var. *muelleri* has resulted from crossing. The parents involved are unknown and quite possibly are no longer existent.

Conclusions

Typha latifolia, when growing beyond the range of *T. angustifolia*, becomes geographically monotypic. A study of the pollen mother cells of this species shows fifteen haploid chromosomes and complete regularity of meiosis, which leads ultimately to an equal distribution of the chromosomes and the formation of only perfect pollen. The latter, when mature, is fully protoplasmic, contains both vegetative and generative nuclei, and is shed in the characteristic "tetrads" of the species.

T. latifolia, when growing in New England, may be "pure," and present cytological features agreeing exactly with those of material isolated from *T. angustifolia*. Close to this, however, are forms which show slightly irregular chromosomal action, and develop a proportion of sterile pollen.

In *T. angustifolia* the number of chromosomes has not been fixed as an invariable quantity, but a larger number appear than were present in *T. latifolia*. The increased number is explained by the presence of univalent chromosomes. These are caused either by disharmony of the parental chromosomes, giving a loose union in

diakinesis and early metaphase stages, or by dissimilarity of the chromosome numbers of the parents. In either case the phenomenon appears to result from hybridity.

Irregular meiotic figures are abundant in *T. angustifolia*, and the small or univalent chromosomes are usually responsible for the lagging. Ordinarily, the tardy action of the univalents leads to the exclusion of a few of these from the tetraspore nuclei. Further abnormality is revealed by the large masses of chromatin formed in some cases (figs. 22, 23), as also by the position of the chromosomes on the spindles. The pollen grains disclose a high degree of malformation and abortion, and at time of shedding only a small percentage show normally protoplasmic conditions.

Forms approaching *T. angustifolia* in taxonomic characters have been found abundant, which, when examined, disclose a large amount of pollen sterility and varying degrees of chromosome irregularity.

The conclusion is reached that *Typha latifolia* shows only the cytological characteristics of a "pure" species in the northern part of its range, but when in proximity to *T. angustifolia* tends to hybridize with it and produce both latifolioid and angustifolioid hybrids.

T. angustifolia seems never to reveal the regularity of meiosis achieved by *T. latifolia*, and develops only a comparatively small percentage of well-formed pollen. It appears probable that this species has arisen through crossing, and now contributes to the further formation of new intermediates through hybridizing with other forms.

T. angustifolia var. *muelleri* shows tetraploidy, and from a comparison with tetraploid species in other genera, such as *Primula*, *Saccharum*, etc., it is believed that this variety of *angustifolia* owes its doubled chromosome number to crossing, either in its remote or recent history.

Summary

1. The fundamental chromosome number of *Typha* is fifteen.

2. When *T. latifolia* grows beyond the range of *T. angustifolia*, the dividing pollen mother cells show normal chromosome distribution, and the pollen consists of uniformly protoplasmic grains.

3. The haploid chromosome number in *T. latifolia* is fifteen.

4. Where *T. latifolia* ranges with *T. angustifolia*, forms appear which show slightly irregular chromosome action and sterile pollen.

5. A larger number of chromosomes appears in *T. angustifolia* than in *T. latifolia;* this is due to the presence of univalents.

6. *T. angustifolia* shows irregularities in meiosis, which consist of lagging, univalent chromosomes, and the frequent exclusion of these from the daughter nuclei.

7. Mature pollen of *T. angustifolia* shows a high percentage of malformed and abortive pollen grains.

8. Intermediates between *T. latifolia* and *T. angustifolia* appear where these two species range together. These show varying degrees of meiotic irregularity, leading eventually to the formation of sterile pollen.

9. The conclusion is reached that *T. latifolia*, when removed from *T. angustifolia*, shows the cytological characteristics of a "pure" species. *T. angustifolia*, however, reveals abnormalities characteristic of hybrids, namely, irregular chromosome distribution and pollen sterility.

10. *T. angustifolia* var. *muelleri* contains thirty haploid chromosomes. Hybridization is offered as a possible explanation of this tetraploidy.

This investigation has been carried on under the supervision of Professor E. C. JEFFREY. I am indebted to him for his suggestion of the study, and for his interest and assistance during its progress.

LABORATORIES OF PLANT MORPHOLOGY
HARVARD UNIVERSITY

[*Accepted for publication March 9, 1927*]

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EXPLANATION OF PLATES XI, XII

Plate XI, *Typha angustifolia* L., pollen formation; ×approximately 2600.

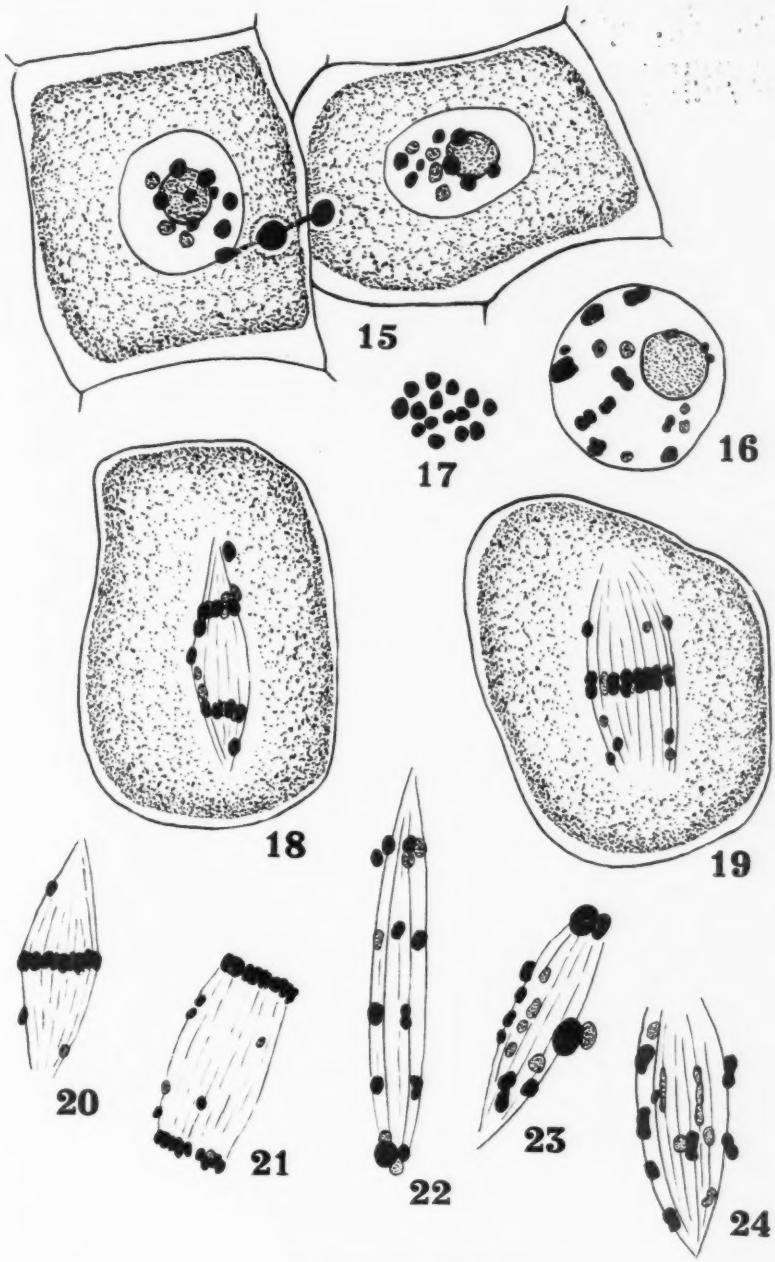
FIG. 15.—Early prophase, showing passage of chromatin-like material from one cell to another.

FIG. 16.—Diakinesis.

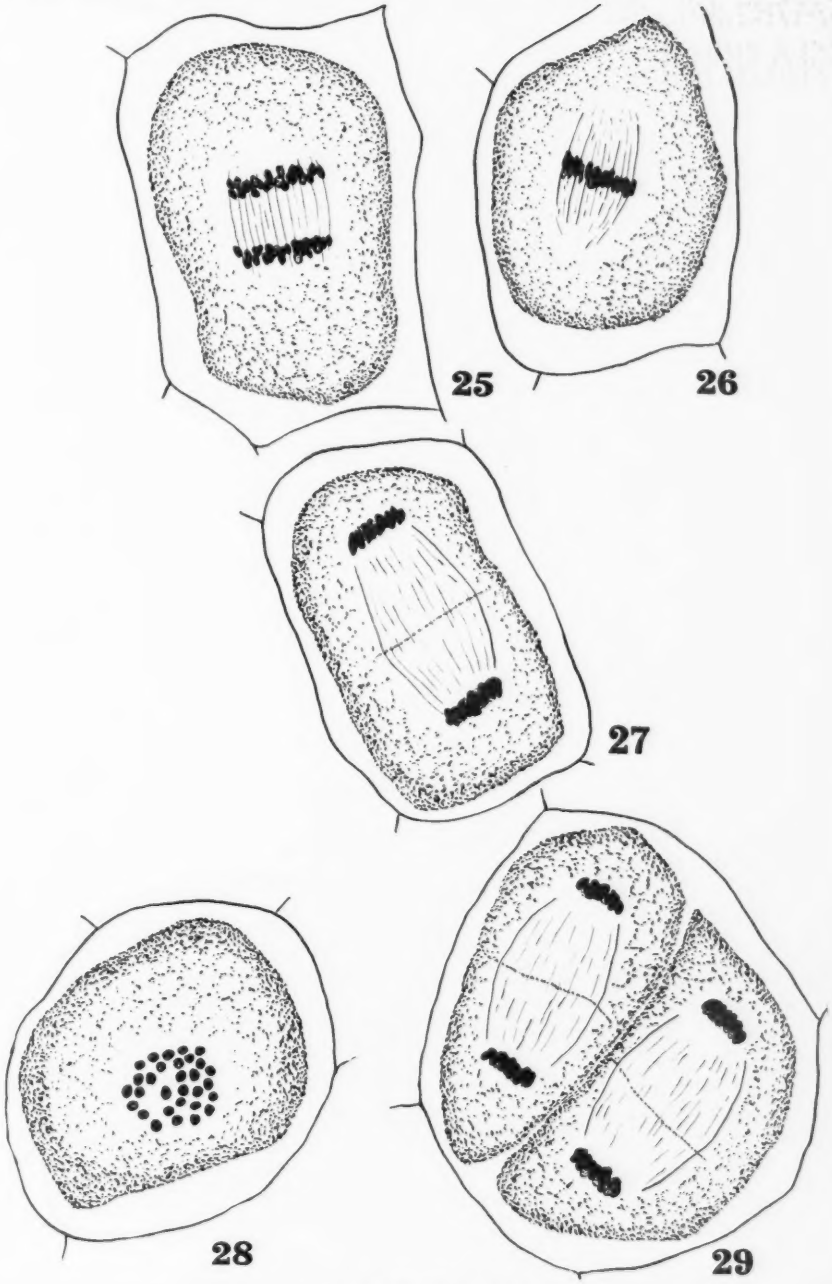
FIG. 17.—Heterotypic metaphase; polar view of a "pseudo-plate."
FIG. 18.—Heterotypic anaphase.
FIG. 19.—Heterotypic metaphase.
FIG. 20.—Heterotypic metaphase.
FIG. 21.—Late heterotypic anaphase.
FIG. 22.—Abnormal elongated spindle in heterotypic division.
FIG. 23.—Early heterotypic metaphase.
FIG. 24.—Early heterotypic metaphase.

Plate XII, *Typha angustifolia* L. var. *muelleri* Graeb., pollen formation; ×approximately 2600.

FIG. 25.—Heterotypic anaphase.
FIG. 26.—Heterotypic metaphase.
FIG. 27.—Late heterotypic anaphase.
FIG. 28.—Heterotypic metaphase, polar view.
FIG. 29.—Late homotypic anaphase.



ROSCOE on TYPHA



ROSCOE on TYPHA

IRON REQUIREMENT FOR CHLORELLA

E. F. Hopkins and F. B. Wann[1]

Introduction

(with three figures)

Although it has been recognized for many years that iron is necessary for the normal development of green plants, very few experimental data on the actual amounts of this element needed to support growth are available. The usual "trace" supplied in most nutrient solutions has always been deemed ample for the plant's needs, and in some cases iron as such is not even included in the formula for the culture solution, it being conceded that enough of the element is supplied as an impurity in the other salts. It is obvious that the amount of iron added to a particular type of nutrient solution as a "trace" will vary directly with the number of investigators employing this solution, while the amounts derived from the other salts as an impurity will depend on the source of the reagents used. Even with iron present in the solution and apparently available to the plant, it is conceivable that it may still be a limiting factor for growth.

Very little is known of the rôle of iron in plant nutrition, although it is generally regarded as being necessary for chlorophyll formation. Various types of chloroses lend support to this idea. Lime-induced chlorosis, extensively investigated by Gile and Carrero (5) and others, appears to be due merely to a depression in the availability of iron in soils with high lime content and consequent low H-ion concentration. In soils of this type the iron is undoubtedly removed from solution both by precipitation and adsorption. In the case also of the manganiferous soils studied by Johnson (8), chlorosis results from the unavailability of iron. Although the reaction of these latter soils is such that ferrous iron would be available

[1] The completion of this joint project was made possible through the cooperation of the National Research Council and Cornell University, to both of which institutions the authors acknowledge their indebtedness.

for plants, this form of iron is readily oxidized by the manganese to the ferric condition, and then precipitated at the H-ion concentration normally characteristic of the soils in question. The relation of the H-ion concentration to the chlorosis of wheat plants has been studied by McCALL and HAAG (11), who found this condition arising in solutions of pH 4.02 to 7. They concluded that the iron was unavailable, or that a faulty metabolism resulted from the immobility of iron in the plant.

It has recently been suggested by ODDO and POLLACCI (13) that iron is necessary for the formation of the pyrrole grouping in chlorophyll, the element in this case functioning as a catalyst. These investigators reported that they were able to obtain normal green plants in solutions supplied with the pyrrole compound but lacking iron. In an attempt to repeat this work, however, DEUBER (4) failed to substantiate their results, but found on the contrary that pyrrole was quite toxic to the several plants used, and apparently could not be substituted for iron in the culture solution.

With reference to the algae, BENECKE (2) states that iron salts act almost entirely as chemical stimulators, and doubts that the necessity of this element can be demonstrated with certainty with algal cultures. Since many of the unicellular green algae can be cultivated on organic media in the dark under pure culture conditions, it would appear that iron may function as a stimulator as in the case of the fungi. The necessity of iron for the latter plants is still an open question. MOLISCH (12) found that on iron-free solutions spore production was inhibited, although iron was apparently unnecessary for vegetative growth. In one of his experiments, however, he obtained practically no mycelium when the iron was completely removed by previous fungus growth. CURRIE (3) states that "iron is not at all necessary for the development of spores" in iron-free cultures of *Aspergillus niger*. The iron-free salts employed by CURRIE were prepared by recrystallization in platinum, but according to STOKES and CAIN (14) all platinum contains a small amount of iron, and it may therefore be questioned whether CURRIE'S solutions were absolutely iron-free. Algae grown in the dark still develop chlorophyll (1), however, and apparently require iron for growth. It would appear that iron plays a rôle entirely apart

from the functioning of chlorophyll in photosynthesis; and its possible connection with respiration, as suggested by WARBURG (17), seems to offer the most promising field for investigation in this connection.

Considerable attention has been directed in recent years to the source of the iron supply in nutrient solutions as affected by the H-ion concentration. GILE and CARRERO (5) studied the assimilation of iron by rice in acid, neutral, and alkaline culture solutions. Ferric chloride they found was practically unavailable in the alkaline cultures, whereas ferric citrate, in the lower concentration employed, yielded just as good growth in the alkaline as in the acid solution. The relation of the H-ion concentration to the availability of iron for *Chlorella* has been pointed out in a recent paper by the writers (7). The unavailability of iron in nutrient solutions at the higher pH values was discussed in connection with the results presented in that paper.

The difficulties involved in obtaining accurate data on the actual amounts of iron needed by the higher green plants is of course evident. The iron content of the seed is always a variable factor, and it would appear to be practically impossible to start with seedlings of no iron content. Securing absolutely iron-free solutions is also difficult. Nevertheless JONES and SHIVE (9) have shown a fairly consistent increase in dry weight of crop with increase in iron content of the solution, indicating that iron may possibly be a limiting factor. The small number of plants used and lack of consideration of the H-ion concentration of the solutions, however, prevent much weight being given their conclusions. In the solutions of very low iron content the plants were chlorotic. In a later paper (10) they have shown that in certain solutions, such as Tottingham's, low yields and chlorosis are associated with high pH values when ferric phosphate is used as a source of iron. This form of iron is available, however, when the solutions contain ammonium sulphate. An explanation of this may perhaps be found in our experiments already mentioned, in which it was shown that a greater absorption of the ammonium-ion from ammonium salts increased the H-ion concentration of the culture solutions. This would tend to make the iron soluble. In general, JONES and SHIVE find that the nature of the

solution with respect to the salt constituents and H-ion concentration appears to determine the availability and efficiency of a given iron salt for plant growth.

In some of their experiments GILE and CARRERO estimate that the amount of available iron must have been less than one part in 10 million of solution. They also obtained larger yields by increasing the amount of iron added to the solutions. Apparently rice was able to assimilate only soluble iron, colloidal iron being unavailable. The necessity of determining the exact amount of soluble iron present in the culture solutions is apparent.

The use of pure cultures of unicellular green algae offers certain distinct advantages in problems of this character. For ease of manipulation, volume of solution required, and exactness of crop determination they are easily superior to higher plants. Moreover, the small amount of inoculum necessary permits of practically complete elimination of any element under study, provided this element can be eliminated from the culture solution. Individual variation is entirely absent where inoculations are made from a uniform suspension of cells from a single stock culture.

In some of our earlier experiments (**7**) on the effect of the H-ion concentration on the availability of iron for the unicellular green alga *Chlorella*, it was observed that in buffered nutrient solutions containing calcium at pH 6 and above, a precipitate of calcium phosphate was produced on which the iron added to the solution was completely adsorbed, even after the addition of sodium citrate. The alga failed to grow in such solutions. The property of adsorption possessed by the calcium phosphate precipitate suggested a method of obtaining a nutrient solution completely free from iron, whereby the necessity of this element for growth, or the actual amounts needed to permit growth, could be studied.

Methods

BASIC IRON-FREE CULTURE SOLUTION.—This solution contained in addition to glucose all the essential mineral elements except iron. Any iron present as an impurity in the compounds used was removed by adsorption on the calcium phosphate precipitate formed

in the preparation of the solution. The following technique was employed.

Two separate lots of one liter each of a solution, designated solution A, were prepared in pyrex flasks of 2-liter capacity. Similar portions of solution B were made up in 3-liter pyrex flasks. The composition of the solutions was as follows:

Solution A		Solution B	
$Ca(NO_3)_2.4H_2O$	5.9 gm.	K_2HPO_4	46.46 gm.
$MgSO_4.7H_2O$	0.8 gm.	Distilled water to	1000 cc.
Glucose	40.0 gm.		
Distilled water to	1000 cc.		

The solutions were sterilized, and when cool one lot of solution A was poured into each of the flasks containing solution B. A heavy precipitate was produced in both mixtures. The mixed solutions were allowed to stand several days, being shaken up a number of times during this interval. The precipitate was then permitted to settle out completely, leaving a clear supernatant liquid. This part of the solution was removed from each of the mixtures by means of a sterile siphon of pyrex glass, the two portions being combined in a single larger sterile pyrex flask. The basic culture solution thus obtained was sterile, was presumably free from iron, and had come in contact with nothing but pyrex glass in its preparation. A test on a portion of the solution showed no iron present.

Standard iron solution.—A standard iron solution was prepared by dissolving 100 mg. of pure iron wire in 5 cc. concentrated HNO_3 to which a little water was added. This solution was evaporated to dryness and the iron taken up in dilute redistilled HCl. This was again evaporated to dryness and the iron finally dissolved in 10 cc. dilute HCl and made up to volume in a 200 cc. volumetric flask. One cc. of this solution contained 0.5 mg. Fe.

Addition of sodium citrate.—As the experiment was originally outlined, it was planned to introduce known amounts of iron (ranging from 0 to 5 mg. Fe per culture) to the various culture flasks, add an equal portion of sodium citrate solution to each, and make up the volume to 25 cc. in all. To these solutions of differing iron con-

tent 25 cc. of the basic iron-free nutrient solution was to be added, making the complete nutrient solution of 50 cc. for each culture.

The use of sodium citrate in conjunction with the standard iron solutions introduced a possible source of iron contamination. Several samples of the salt were tested for iron, and all contained considerable quantities; hence the addition of the salt to the standard iron solutions would introduce an error in the actual amounts of iron added, and the check cultures, receiving sodium citrate but no iron, would in reality receive iron as an impurity in the citrate.

To overcome this difficulty, the possibility of adding the sodium citrate to solution A in order to remove the iron from this salt by adsorption on the calcium phosphate precipitate was investigated. It was found, however, that the citrate was apparently carried down with the precipitate to such an extent that the resulting clear liquid would not subsequently hold iron in solution at pH 7.5. In this connection it was also determined that iron adsorbed by the calcium phosphate precipitate could not readily be brought back into solution by the addition of more sodium citrate.

The only alternative was to obtain sodium citrate free from iron. This was done by recrystallization. A considerable quantity of C.P. salt ($2Na_3C_6H_5O_7 \cdot 11H_2O$) was recrystallized twice from distilled water, and finally from conductivity water in pyrex glass vessels. The resulting salt consisted of very minute crystals and was practically free from iron. A portion of the salt dried at 90° C. showed 78 per cent sodium citrate.

In previous experiments it was found that 0.01 gm. of sodium citrate was sufficient to hold 0.05 mg. of iron in solution in each 50 cc. culture. In the present series, however, the use of larger amounts of iron was planned, and it was also realized that some calcium would be present in the basic iron-free nutrient solution which might affect the solubility of iron. To test the ability of the recrystallized sodium citrate to hold 5.0 mg. of Fe in solution in the complete culture medium, the following trial was made. To 10 cc. of standard iron solution (5 mg. Fe) 0.5 cc. of a 2 per cent sodium citrate solution (0.01 gm. Na citrate) was added in a small Erlenmeyer flask. Solutions A and B were prepared and mixed, the usual precipitate of calcium phosphate forming. The clear iron-free solu-

tion was obtained by centrifuging and adding 50 cc. to the iron-sodium citrate solution. A precipitate resulted. It was evident that the iron was thrown out of solution. Further tests were then made with increasing amounts of citrate added to 10 cc. of standard iron solution. In each case the volume was brought up to 20 cc. with water, and 50 cc. of the iron-free nutrient solution then added. It was found that 1.5 or 2 cc. of the 2 per cent sodium citrate solution would prevent the precipitation of 5 mg. Fe under these conditions. Thus by using 0.04 gm. sodium citrate per culture, it was possible to hold the largest amount of iron added (5 mg.) completely in solution with all the other salts of the complete nutrient solution present and at a pH of approximately 7.5.

PREPARATION OF SOLUTIONS OF DIFFERING IRON CONTENT.—About 75 pyrex Erlenmeyer flasks of 150 cc. capacity were thoroughly cleaned and drained. To each of these (except for solutions nos. 1 and 2) 1 cc. of a 4 per cent citrate solution (0.04 gm.) was added, using a pipette of pyrex glass. Iron was then added to these in groups of four for each concentration, as shown in table I. The total volume was brought up to 25 cc. in each of the culture flasks by the addition of the necessary amount of redistilled water, as indicated in the table, allowing for 1 cc. of sodium citrate solution in all cultures except with solution nos. 1 and 2, in which cultures it was proposed to omit both iron and sodium citrate. The culture flasks were then sterilized at 15 lb. for 30 minutes.

The sterile basic iron-free culture solution was then added to the culture flasks in 25 cc. quantities by means of sterile pipettes. The final volume of each complete culture solution was thus 50 cc. The original concentrations of solutions A and B, as presented earlier, were reduced to one-fourth these values by the two combinations involved in the preparation of the final culture medium.

Three flasks of each different concentration of iron, three "blanks" in pyrex flasks, and three "blanks" in quartz flasks (no citrate and no iron) were then inoculated with a suspension of *Chlorella* cells. The date of inoculation was January 5, 1926. The suspension was prepared in a portion of the sterile iron-free solution, a pure culture of the organism on mineral nutrient agar supplying the cells. The only iron introduced with the suspension was that

present in the cells themselves. Each inoculated culture received 0.5 cc. of this suspension. The remaining uninoculated flasks served for initial iron and pH determinations.

Conductivity water in pyrex flasks showed no iron after long sterilization, so that the possibility of iron dissolving from the pyrex glass is remote. With the exception of some of the pipettes, all glassware used was of pyrex.

TABLE I

COMPOSITION AND INITIAL IRON CONTENT OF NUTRIENT SOLUTIONS; EXPERIMENT 1

Solution no.	Cubic centimeters of iron standard no.			Redistilled H_2O (cc.)	4% Na citrate solution (cc.)	Basic Fe-free solution (cc.)	Mg. Fe per culture	
	1	2*	3†				Added	Found
1.....	0.0	0.0	0.0	25.0	0	25	0.0000	Blank‡
2.....	0.0	0.0	0.0	25.0	0	"	0.0000	Blank
3.....	0.0	0.0	0.0	24.0	1	"	0.0000	Blank
4.....	0.0	0.0	0.2	23.8	1	"	0.0001	.....§
5.....	0.0	0.0	0.4	23.6	1	"	0.0002	——
6.....	0.0	0.0	0.8	23.2	1	"	0.0004	——
7.....	0.0	0.0	1.0	23.0	1	"	0.0005	——
8.....	0.0	0.0	2.0	22.0	1	"	0.0010	0.001‡
9.....	0.0	0.0	4.0	20.0	1	"	0.0020	0.0015
10.....	0.0	1.0	0.0	23.0	1	"	0.0050	0.0065
11.....	0.0	1.4	0.0	22.6	1	"	0.0070	0.0075
12.....	0.0	2.0	0.0	22.0	1	"	0.0100	0.01
13.....	0.0	4.0	0.0	20.0	1	"	0.0200	0.02
14.....	0.0	6.0	0.0	18.0	1	"	0.0300	0.03
15.....	0.0	10.0	0.0	14.0	1	"	0.0500	0.05
16.....	0.2	0.0	0.0	23.8	1	"	0.1000	0.1‡
17.....	1.0	0.0	0.0	23.0	1	"	1.5000	0.5‡
18.....	2.0	0.0	0.0	22.0	1	"	1.0000	1.0
19.....	10.0	0.0	0.0	14.0	1	"	5.0000	5.0

* 10 cc. of iron standard no. 1 diluted to 1000 cc. = 0.005 mg. Fe per cc.

† 1 cc. of iron standard no. 1 diluted to 1000 cc. = 0.0005 mg. Fe per cc.

‡ In fused quartz glassware; all other cultures in pyrex flasks.

§ The amounts of iron in the 10 cc. samples of solutions 4 to 7 inclusive were below the limit of the colorimetric test employed.

DETERMINATION OF INITIAL IRON CONTENT OF CULTURE SOLUTIONS.—Standard iron solutions were prepared in 50 cc. Nessler comparison tubes, using the method previously described (7). HCl redistilled through a pyrex condenser and measured with a pyrex pipette was employed. Fresh KSCN solution was used. The original iron wire solution was used for the more concentrated iron standards, and fresh dilutions of this stock were prepared for the lower iron concentrations. This is necessary because in dilute solution the iron

is apparently adsorbed on the walls of the volumetric flasks after standing for some time, and such solutions do not give true values for iron.

A blank determination on the reagents (HCl, KSCN, H_2O, and acetone) showed a trace of color when compared with a tube of water. The standard containing 0.0002 mg. Fe, however, could be distinguished from the reagent blank with certainty, although this appears to be the lower limit of the test. Differences between standards containing 0.0002 and 0.0003 mg. Fe were readily distinguished.

Ten cc. from each of the uninoculated culture solutions were now tested for iron by comparison with the preceding standards. These results are included in table I. It will be noted that the amounts of iron found are in close agreement with the calculated amounts added.

At the same time determinations of the initial H-ion concentration of the various solutions were made, using the GILLESPIE colorimetric method (**6**). Except for the solution containing 5 mg. Fe, the pH values were quite uniform throughout. This one exception can be accounted for by the addition of more free HCl with standard iron solution.

OBSERVATIONS ON GROWTH OF CULTURES.—One week after inoculation only a trace of growth was observed, this being confined to the cultures containing the higher concentrations of iron. This limited growth of the alga was conceivably due to the unavailability of nitrogen, as proposed in connection with Experiment 6 in the series of studies on the effect of pH on the growth of *Chlorella* (**7**). It was thought that the addition of NH_4NO_3 to the cultures might permit of more rapid growth. Accordingly on January 12, 1926, 1 cc. of sterile NH_4NO_3 solution containing 0.025 gm. per cc. was added to one culture of each iron concentration. The remaining cultures were not changed. It will be seen from the dry weights of the crops, however, that the addition of NH_4NO_3 was apparently without any effect on the growth of the organism.

Results of first experiment

On January 30 the following observations were made. Good growth was present in cultures with 5 and 1 mg. Fe, the color in

both lots being dark green. A rather copious flocculent precipitate was present in the first mentioned lot. At 0.5 mg. Fe there was very fair growth, but light green in color; at 0.1 mg. Fe, fair growth but brownish in color; at 0.05 mg. Fe, growth slight and color yellowish; at 0.03 and 0.02 mg. Fe, a trace of growth. In the remaining cultures there was no growth.

At this time the algal crops were harvested and the dry weights determined in the manner described in our earlier papers. Final pH

TABLE II

GROWTH OF CHLORELLA IN NUTRIENT SOLUTIONS WITH DIFFERING IRON CONCENTRATIONS; EXPERIMENT 1

SOLUTION NO.	PH OF SOLUTION				FE IN MG. PER CULTURE				DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
	Initial control	Final			Initial control	Final						
		A	B	C		A	B	C	A	B	C	
8*	7.1	.....	.....	.....	0.001+	.....	.....	.....	.....	.....	.....	.....
9	7.1	.....	.....	.....	0.0015	.....	.....	.....	.....	.....	.....	.....
10	7.1	.....	.....	.....	0.0065	.....	.....	.....	.....	.....	.....	.....
11	7.1	.....	.....	.....	0.0075	.....	.....	.....	.....	.....	.....	.....
12	7.1	7.4	7.4	7.4	0.01	0.0129	0.0106	0.0082	0.4	1.2	2.5	1.37
13	7.1	7.4	7.4	7.4	0.02	0.0215	0.0210	0.0210	1.0	1.7	4.3	3.33
14	7.1	7.4	7.4	7.4	0.03	0.0364	0.0315	0.0252	3.0	1.7	6.7	3.80
15	7.1	7.3	7.4	7.4	0.05	0.0451	0.0462	0.0420	3.7	12.0	16.0	10.57
16	7.2	7.3	7.4	7.4	0.1+	0.0696	0.1075	0.1275	19.9	10.2	26.6	18.90
17	7.2	7.2	7.4	7.3	0.5+	0.3440	0.4200	0.4200	70.1	76.5	82.2	76.37
18	7.1	Lost	7.4	7.2	1.0	Lost	0.8500	0.8300	Lost	82.3	104.4	93.35
19	6.5	6.6	6.8	6.7	5.0	0.0033	0.0033	0.0033	80.2	83.6	78.4	80.27

* There was no growth in solutions 1 to 11 inclusive. Included in this table are those solutions only for which determinations of initial iron content would be made with certainty. The initial pH of solutions 1 to 7 inclusive was 7.1 in each case.

and final iron determinations were made on the residual culture solutions. The data are presented in table II and fig. 1. It will be observed that the best growth was obtained with the higher iron concentrations, 5 and 1 mg. Fe per culture, although growth was nearly as good at 0.5 mg. Fe. Below this the growth was dropped sharply to zero at 0.007 mg. Fe per culture. The final pH determinations showed that all the solutions tended to become more alkaline, due probably to the utilization of NO_3 ions in excess of Ca or K. The final iron tests indicated that comparatively little iron was taken up by the alga during growth. In the lower concentrations of iron, where the alga crops were small, almost complete recovery of the original iron was recorded. In the cultures containing 5.0 mg. Fe

there was only the slightest trace of iron left in the solution, the bulk of it being precipitated.

Results of second experiment

The results of the first experiment, although consistent and apparently representative of the prevailing conditions, seemed to contradict our previous experience with the growth of *Chlorella* in several different nutrient solutions. In many former experiments only 0.02 mg. Fe per culture was actually added to the solutions, although this

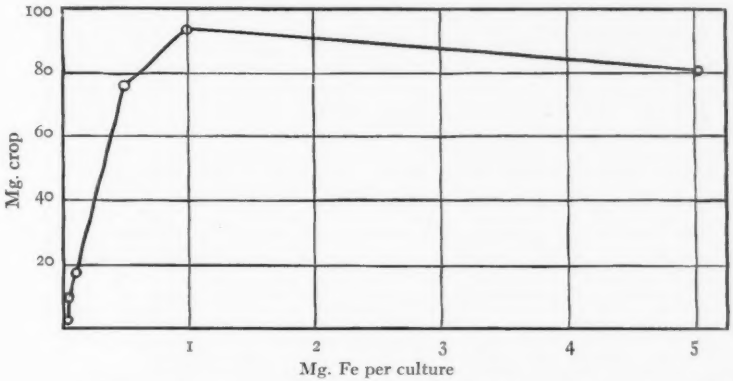


FIG. 1.—Effect of concentration of iron on growth of *Chlorella;* Experiment 1

amount was usually increased to about 0.05 mg. per culture by the iron present as an impurity, principally in the glucose. With this amount of iron, crops of 80–100 mg. were frequently produced in two weeks. In the experiment just described, however, such crops were attained only where at least ten times that amount of iron was present. In Experiment 6 of our earlier paper crops of over 100 mg. were produced on a solution with an initial iron content of 0.05 mg. per culture, and an initial pH of 7.5. In this case, however, calcium was omitted from the solution, whereas the solutions with differing iron content may have contained some calcium as a constituent of the basic iron-free nutrient solution. It is possible that an altered permeability of the cells to iron may have been produced by the calcium, thus explaining in part the apparently higher iron requirement in the latter solutions.

In the first experiment, just presented in detail, the cultures were maintained in the laboratory, where during the winter light may have been an important factor in the amount of growth obtained. It was decided, therefore, to repeat the experiment in the spring, growing the cultures in the greenhouse. The procedure adopted was exactly the same as in the earlier series, and all the reagents used in the preparation of the basic iron-free nutrient solution were from

TABLE III

GROWTH OF CHLORELLA IN SOLUTIONS OF DIFFERING IRON CONTENT; EXPERIMENT 2

SOLUTION NO.	FE ADDED MG. PER CULTURE	pH OF SOLUTIONS				FE IN CONTROL (MG.)	DRY WEIGHT OF CROP (MG.)			AVERAGE CROP (MG.)
		Initial control	Final							
			A	B	C		A	B	C	
1	0.000	7.3	7.3	7.3	7.3	0.000	.....	.....	.....	.....
2	0.010	7.3	7.3	7.3	7.3	0.025	.....	.....	.....	.....
3	0.040	7.3	7.3	7.3	7.3	0.055	.....	.....	.....	.....
4	0.050	7.3	7.3	7.3	7.3	0.079	.....	.....	.....	.....
5	0.075	7.3	7.3	7.3	7.4	0.092	0.2	0.0	1.0	0.26
6	0.100	7.4	7.3	7.2	7.3	0.115	2.8	76.7*	12.5	5.10
7	0.200	7.3	7.3	7.3	7.3	0.276	44.6	35.6	50.9	43.7
8	0.300	7.3	7.2	7.3	Cont.	0.368	99.0	75.9	Cont.	87.4
9	0.400	7.3	7.2	Cont.	7.2	0.552	74.9	Cont.	82.0	78.4
10	0.500	7.3	7.1	7.2	7.1	0.736	Lost	86.5	90.8	88.6
11	0.750	7.3	7.1	7.1	7.1	0.920	96.3	99.6	104.1	100.0
12	1.000	7.3	7.1	6.9	6.9	1.150	108.9	105.6	115.7	110.1
13	2.500	7.1	5.9	6.9	6.8	2.250	133.8	141.5	151.2	142.2
14	5.000	6.3	Cont.	6.7	6.5	4.600	Cont.	107.0	120.4	113.7
15	10.000	5.4	5.4	5.4	5.4	0.0125	002.5	001.6	003.8	002.6

* Omitted from average. The heavy crop in this case probably resulted from accidental iron contamination.

Cont. = culture contaminated.

the same reagent bottles. A new standard iron solution was prepared however.

STANDARD IRON SOLUTION.—This was made by dissolving 500 mg. of pure iron wire in dilute (1–9) redistilled HCl. This solution was saturated with chlorine washed through concentrated H_2SO_4. The solution was then evaporated to dryness on the water bath. The iron was taken up in 15 cc. redistilled HCl to which a little water was added, and the solution finally diluted to 1000 cc. in a pyrex volumetric flask. One cc. of the standard solution contained 0.5 mg. Fe.

SODIUM CITRATE SOLUTION.—In this experiment a higher concentration of iron (10 mg. Fe per culture) was introduced into the series. It was therefore necessary to increase the dose of sodium citrate in order to hold this amount of iron in solution. For this purpose 3 cc. of a 5 per cent solution of the recrystallized salt was added to each culture flask.

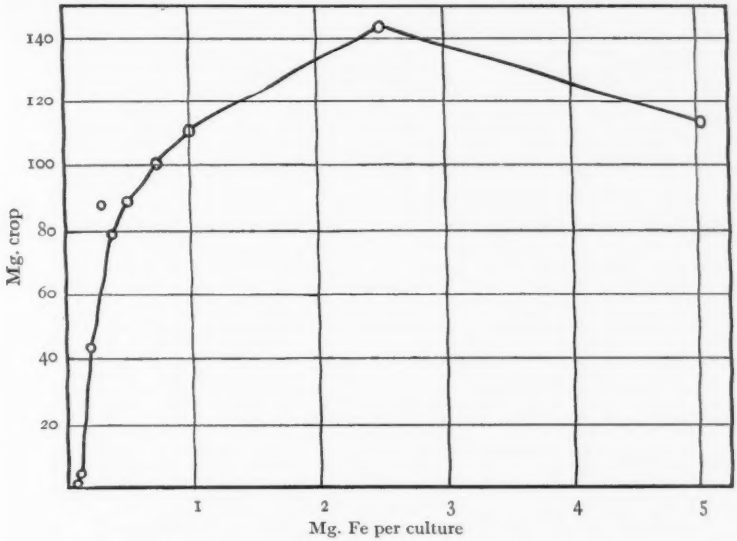


FIG. 2.—Effect of concentration of iron on growth of *Chlorella;* Experiment 2

PREPARATION OF SOLUTIONS OF DIFFERING IRON CONTENT.—Pyrex Erlenmeyer flasks of 150 cc. capacity served as culture containers. Following the addition of the sodium citrate solution to each flask, iron was added to each of four flasks for each of the concentrations given in table III. The volume was brought up to 25 cc. in each flask by the addition of the necessary amount of distilled water. The flasks were then sterilized.

After the introduction of 25 cc. of sterile basic iron-free nutrient solution to each of the culture flasks, three of each iron concentration were inoculated with a suspension of *Chlorella* cells, the fourth in each case being reserved as a control for pH and iron determina-

tions. The cultures were inoculated on June 21, 1926, and immediately placed in the greenhouse under partial shade.

At the end of two weeks no growth was observed in any of the first four groups of cultures, with iron content ranging from 0 to 0.05 mg. Fe per culture. In the remaining cultures the amount of growth apparently increased markedly with an increase in the iron content, from a "trace" in the solution with 0.075 mg. Fe to a very good growth in solutions with 1, 2.5, and 5 mg. Fe per culture. In the highest concentration of iron (10 mg.) no growth could be detected, but a heavy flocculent precipitate was present. When this precipitate was dissolved with a little HCl, it was found that the organism had made a slight growth in this solution.

Crop determinations were made in the usual way on July 6. Determinations of the final pH and iron content of the culture solutions were made at this time, as well as analyses of the "control" solutions. The data are presented in table III, and the relation of amount of growth to iron concentration is shown graphically in fig. 2.

Discussion

In the preceding experiments advantage was taken of the fact brought out in our previous work that iron can be almost completely removed from solution in certain culture media by adsorption on the calcium phosphate precipitate formed in alkaline solutions. It is probable that the high concentration of the phosphate ion in our solutions also aided in decreasing the solubility product for both $Ca_3(PO_4)_2$ and $FePO_4$. The method is suggested as a convenient means of freeing culture solutions from iron. Once having this iron-free solution, one may then proceed to study the effect of known amounts of iron added to the cultures on the growth of the organism under consideration.

Previous methods have usually depended upon the careful purification of the water, individual salts and organic components which enter into the composition of the culture medium by recrystallizations, redistillations, etc. (see MOLISCH **12** and CURRIE **3** for examples of this procedure). Objections to this method might be brought up; first, slight amounts of iron in the component parts of the solution which remain even after purification will be added

together when the solution is prepared; second, manipulation of the purified reagents would allow chance for contamination with iron.

Using the adsorption procedure, it was found that the iron was removed to such an extent that it could not be detected by our method of determination, and also so that there was insufficient iron for growth of *Chlorella*. Although, as will be shown later, the minimum iron concentration for this organism has not been found, it is thought to be low. Several difficulties present themselves in the use of such an iron-free solution for the investigation of the effect of iron concentration. The adsorption is of necessity carried out at an alkaline reaction, and if an iron salt is then added in varying amounts to portions of the iron-free solution it is rapidly precipitated as $FePO_4$. If one wishes to overcome this difficulty by shifting the reaction to a more acid one, he may possibly add iron in the acid used. And granted that this is done with an iron-free acid, iron will precipitate slowly at most acid reactions which are at pH values above the acid limit for growth. The iron would therefore be variable in each of the cultures during the course of the experiment, and the results would have no meaning.

In our work we attempted to obviate this difficulty by adding sodium citrate to the culture solutions. This we knew from our previous experiments (7) would keep the iron in solution, even at alkaline reactions. When the sodium citrate was added to the culture solution before adsorption of the iron impurities, however, it was found that it was also removed to a large extent by adsorption. When added afterward there was, of course, the possibility of adding some iron impurity which might affect the results at the low iron concentrations. The principal objection to the use of this salt may be its effects on the dissociation of the iron salt. This will be discussed later.

The change in color in iron solutions when an excess of sodium citrate is added to them seems to show that the iron changes into another form. It is usually stated that a complex organic salt of iron forms. What occurs might be considered to take place as follows: the addition of the sodium citrate to a ferric chloride solution of a pale straw color changes it to yellow, probably the color of the molecular form of ferric citrate. An excess of the citrate deepens

the color by decreasing the dissociation and giving more of the molecular form. This would be analogous to the effect of an excess of KSCN on the ferric sulphocyanate reaction. The iron apparently is true solution and not in a colloidal state, for it is not precipitated in the presence of the sulphate and phosphate ions which are in the culture solutions. That the sodium citrate does not act as a protective agent is evident from tests made by the writers that showed colloidal iron solutions to be precipitated by sulphate and phosphate ions, even in the presence of sodium citrate.

Both the high amount of iron present at the lower limit for growth and the large amount necessary for good growth in these experiments are hardly in keeping with results of other workers or with our previous experiments. Taking the results as they stand, however, in Experiment 1 we have a rapid increase in growth as the amount of iron increases from the limiting concentration of 0.0075 to 1 mg. per culture. An optimum concentration is not well defined, but at 5 mg. per culture there is a falling off in the growth. In Experiment 2 the results are similar; the curve rises rapidly from a limiting concentration, in this case of 0.075 mg., to an optimum concentration at about 2.5 mg. per culture, and drops again at 5. At 10 mg. there is practically no growth, but this is due to loss of iron by precipitation, as the table shows, and is probably not due to toxicity. There was too little sodium citrate to keep this large amount of iron in solution. Whether the smaller crops in both experiments at 5 mg. of iron per culture is the result of toxicity is uncertain, but it should be pointed out that in both cases there was a distinct change in the reaction of the culture medium caused by the amount of acid added with the standard iron solution, in spite of the fact that the culture solutions were strongly buffered. The conditions involved are rather complicated, and it will require further study to elucidate matters.

One thing difficult to explain has been the difference between the amount of iron needed for good growth of *Chlorella* in the present work and in our previous experiments (**7**) on the effect of pH on growth. In the latter experiments, as has already been mentioned, good crops of the alga were obtained at alkaline reactions when only 0.05 mg. of iron per culture (50 cc.) was used; for example at pH

7.15, 83.4 mg. were obtained (see Experiment 6 of that paper), while in the present experiments very little growth was obtained at this concentration, namely, 10.6 mg. in Experiment 1 (pH 7.1–7.4), and none in Experiment 2 (pH 7.3). It was at first thought that the difference might be explained by the fact that a calcium salt was used in the preparation of the culture solutions in the second case and not in the first, and that the presence of calcium might either decrease the ionization of the iron salt in some manner, or more likely change the permeability of the cells to iron. This is probably not true unless the calcium acts in very minute amounts to produce this effect, because a rather sensitive qualitative test failed to show the presence of calcium. It had apparently precipitated out quite completely as the phosphate in the preparation of the iron-free solution.

Since performing these experiments and writing most of this paper, some investigations by USPENSKI which have been called to our attention seem to give a better explanation of the differences found. USPENSKI (**15**) considers that when certain organic substances, such as sodium citrate (or tartrates as in Raulin's medium), are present in culture media the iron enters into complex organic combination, and in such state is only slightly ionized and hence only available in low concentration. It might appear therefore that large quantities of iron were necessary for growth under these conditions, while actually only a small amount of what is present is active. The solution acts as a buffer as regards iron. USPENSKI also shows that the addition of citrate markedly decreases the intensity of the reaction with KSCN. If, for example, at pH 3.4 the reaction of a given iron solution without citrate is taken as 100, the addition of 0.000005 M citrate decreases the intensity to 80 and of 0.00005 M citrate to 25. In another paper USPENSKI and USPENSKAJA (**16**) state that in the presence of 0.004 M sodium citrate the most favorable concentration of iron for *Volvox* is from 2.5–5 mg. Fe_2O_3 per liter, while in the pure nutrient solution without citrate 0.5–1 mg. Fe_2O_3 per liter is best. The pH was 7.6. The important and interesting idea they put forth is that it is not the total amount of iron present but the amount of iron-ion which is active.

In our experiments on iron greater amounts of sodium citrate

were used than in those concerned with the effect of the H-ion, and in the second experiment more citrate was added than in the first, and it is possible in these cases that because of the high citrate content we had decreased the Fe-ion concentration to a low value, although the total amount of iron was large. In Experiment 6 of

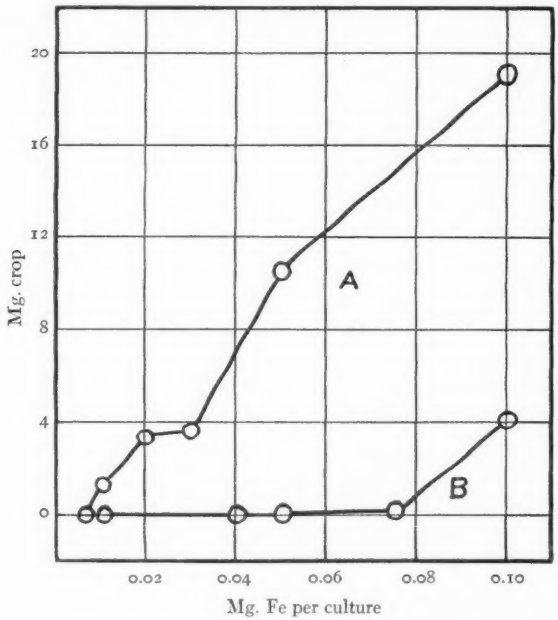


FIG. 3.—Limiting concentrations of iron for *Chlorella* plotted to larger scale: *A*, Experiment 1 in which low concentration of sodium citrate (0.04 gm. per culture) was used; *B*, Experiment 2 in which high concentration (0.15 gm. per culture) of sodium citrate was used.

our first paper 0.01 gm. of sodium citrate per culture was used. In Experiments 1 and 2 of this paper 0.04 and 0.15 gm. respectively were used. This appears therefore to explain the large differences found. Fig. 3, in which the data for Experiments 1 and 2 for small amounts of iron are graphed on a larger scale, shows strikingly this difference. The limit for growth with the greater amount of citrate

is at 0.075 mg. of iron per culture, with the smaller amount at 0.0075, or about one-tenth as much.

These results lend support to the ideas of USPENSKI that it is not the total amount of iron in the culture medium, but the amount in the ionized form which is effective physiologically. The iron is of course assumed to be in solution. Further, our results do not show a limiting and an optimum iron concentration for growth of *Chlorella*, but only the limiting and optimum concentrations for a given set of conditions. Indeed, will it be possible to determine this except for particular iron salts in given culture media? The effect of the iron-ion would give perhaps more consistent results. The case is analogous to the effect of total acidity and of H-ion concentration on plant growth. It is probable, however, from the results of these experiments, although no knowledge is at hand in regard to the extent of ionization in the solutions used, that the iron-ion concentration for good growth of *Chlorella* is small. It is interesting to note that although this may be true, we are able to remove the iron by adsorption below the limit where growth will occur. This is shown in these experiments by the absence of growth in the "iron-free" nutrient medium to which was added neither sodium citrate nor iron.

Summary

1. There are no fundamental data on the iron requirements of plants. This is true of both minimum and optimum amounts. This lack of information is due in some cases to failure to consider the solubility of the iron added to culture solutions, and in others to failure to remove completely all the iron present as impurities in such solutions.

2. We have presented a method of removing from culture solutions apparently the last traces of iron by means of adsorption at alkaline reactions, and have further shown that if sufficient sodium citrate is used in solutions free from precipitate, iron added subsequently will remain in solution indefinitely.

3. In an attempt to use these methods to determine the relation of iron to the growth of *Chlorella*, a high minimum concentration was found which varied in the different series, and was higher in those which had a higher sodium citrate content.

4. The hypothesis put forth in explanation of these results is that iron is active in growth only in the ionized form, and that increasing the amount of citrate decreases the ionization of the ferric citrate present in these solutions. Therefore, while we may have a larger total amount of iron present, we may have little or no growth because of a low concentration of the ferric ion. This explanation is in keeping with the work of USPENSKI.

LABORATORY OF PLANT PHYSIOLOGY
CORNELL UNIVERSITY

[*Accepted for publication May 17, 1927*]

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ECOLOGY, PLANT GEOGRAPHY, AND GEOBOTANY; THEIR HISTORY AND AIM

EDUARD RÜBEL

Introduction

Are ecology, plant geography, and geobotany three different sciences, or three different viewpoints of the same science? Is one a part of the others, or are all three synonymous? Opinions on these questions differ widely, and the writer thinks it worth while to review the history and discuss the matter. The necessity of discussing it again was emphasized at the Ithaca Congress. It was evident there that ecology is often understood in a rather narrow sense by those not especially interested in it; while American ecologists use it in a very broad sense, applying the term to all their work and intending it to embrace also chorological, chronological, and genetical phases of botany and zoology. Reducing the term to a narrow meaning when it is meant in a broad one brings it into a somewhat wrongly isolated position, especially unwelcome in our times of over-specialization. To correct misunderstandings by non-ecologists, I wish to discuss what ecology includes in the minds of ecologists, what plant geography means to plant geographers, and what geobotany comprehends, giving also the other narrower sense of each term.

Ecology,[1] from *oikeo* to dwell, can include all science about dwelling, environment, household. The whole earth, with its climates, soils, and bions, is environment to the plant or the plant community; so that the study of all factors, static and dynamic, and all the regional influences on all beings distributed over the earth is ecology: plant ecology, and animal ecology including human ecology or ethnography.[2]

Geography is the study of the earth, of its inorganic part in

[1] The term originated from HÄCKEL in his *Generelle Morphologie der Organismen*, 1866, as the science of relationships of organisms to environment (Wissenschaft von den Beziehungen der Organismen zur Aussenwelt).

[2] The section of Ecology in the Congress of Plant Sciences was meant in this wide sense, but four such ecological papers were read at the same time in the section of Taxonomy.

physical and morphological geography, of its organic part in plant, animal, and human life, especially economic geography. One must consider how and why the beings can and do live on the earth, how they must be distributed, and how they react; if the beings under consideration are plants or plant communities, the study is plant geography.

Geobotany is that part of botany which has to do with *gea*, the earth, the action of all earthly factors, all changes of plants on the earth, all distributional questions over the earth. GRISEBACH (6) wrote as long ago as 1866 "Die auf die Geographie der Pflanzen gerichteten oder, wie man sich kürzer ausdrücken kann [not only shorter but I should say more precisely and accurately], die geobotanischen Forschungen haben die Aufgabe," etc. Later on he discusses "Allgemeine Geobotanik" and "Spezielle Geobotanik, die Vegetation der einzelnen Länder." Everyone will admit that taken in this broad sense the three terms are synonymous.

Yet other synonyms of our science are "history of plants"[3] WILLDENOW 1792 (23), and "phytostatic" THURMANN 1849 (20). WILLDENOW gives the following definition:

Unter Geschichte der Pflanzen verstehen wir den Einfluss des Klimas auf die Vegetation, die Veränderungen, welche die Gewächse wahrscheinlich erlitten haben, wie die Natur für die Erhaltung derselben sorgt, die Wanderungen der Gewächse und endlich ihre Verbreitung über den Erdball.

And THURMANN writes:

La géographie botanique présente le tableau des faits de dispersion végétale et les met en rapport avec leurs causes. L'expression de phytostatique qui a déjà été employée par quelques observateurs satisfait bien à cette définition. Elle est plus générale et plus exacte que celle de géographie botanique qui, en éveillant particulièrement l'idée descriptive dans de grandes proportions, n'implique aussi bien, ni le point de vue topographique, ni le côté spéculatif de l'étude stationelle.[4]

Le sens essentiellement stationel que l'on a attaché au mot phytostatique est donc en réalité tout-à-fait légitime et conforme à l'étymologie.

So these two terms mean the same thing as the three we are principally considering. What do they embrace within botany?

[3] "History" is nowadays still often used in the sense of description (story from *storia*, a late Latin derivation of the ancient Latin *historia*). Also "natural history" comprises the whole science of nature, not only a historical portion of it.

[4] Phytostatique includes station, habitat (climate and soil).

Historical and logical branches of biology

The beginning of pure botany was a general description and classification of the exterior of plants. Thus these are the beginnings of morphology and taxonomy. Later on arose the questions about life-processes, physiology. When the microscope was invented, morphology was enlarged by anatomy, the study of the micro-forms seen after making cut sections (*anatemno* to cut). For a long time these three phases of botany prevailed. Much later geographical, ecological, palaeontological, and genetical questions arose, and grew to sections of plant sciences of equal standing.

Instead of this purely historical sequence of branches of botany, TSCHULOK (**21**) gave a logical one considering what is necessary and sufficient ("notwendig und hinreichend") to cover all physical knowledge about living beings. There are seven incommensurate material points of view.

1. Taxonomy, the science of relationship: the distribution of the plants in groups according to their degree of similarity. These are species, genera, families, etc.

2. Morphology, the science of form: the study of the outer and inner (anatomical) form.

3. Physiology, the science of the processes of life.

4. Ecology, the science of the household: the adaptation of organisms to the external world, the earth; the arrangement of the household.

5. Chorology, the science of space: the distribution of the organisms in space (on the earth).

6. Chronology, the science of time: the chronological appearance of the organisms in earth history.

7. Genetics, the science of development: the descent of organic beings.

Our science is not one of these. It has a compound point of view, and may be defined as follows:

Geobotany (plant ecology—plant geography) is the science of the relationship of plants to the environment, the earth.

Several of the seven points fall into this definition. Morphology and physiology concern form and function of the single plant and its organs, and thus can be considered without necessarily knowing the

environment. Taxonomy groups the *Sippen* without necessary relation to surrounding land, but chorology asks how plants are distributed on the earth and how they migrate; ecology endeavors to determine how plants arrange their household on the earth within the given climate, soil, and conditions of competition. Chronology considers the bearing of the plant to time, to earth periods. This chronological or historical viewpoint is intimately connected with genetical evolution. The earth periods in which the plants grow are subject to change; at the same time the plants themselves change under these acting forces. The geogenetical question of chronology is always united with the phylogenetical one of genetics proper, and forms evolutionary botany.

Three groups of problems

Since environment acts in different directions and postulates different questions, we must distinguish in geobotany three great groups of problems:

1. The problem of space, the question of the distribution of plants on the earth.

2. The problem of habitat, the organization of the household of plants, the question of behavior toward habitat in the widest sense.

3. The problem of change, the question of plant behavior toward the changes of the earth and the changes of the plants themselves in time.

Of course the different problems must not always be considered separately; on the contrary in any paper all have to be kept in mind, even when one of them predominates in the study. Often they combine necessarily. In the historical consideration of Tertiary or Pleistocene plants there is also a problem of space and habitat. Although we treat the Pleistocene and Tertiary plants in genetical (historical) geobotany, because they lie far behind in the genesis of the earth, there nevertheless exists a chorological geobotany of the Pleistocene, an ecological geobotany of the Pleistocene.[5]

[5] In the lecture programs of the University of Zürich one finds that in 1911 and several times in later years courses were announced: BROCKMANN-JEROSCH: Pflanzengeographie des Diluviums (an extension of the field in accordance with COOPER'S wishes of 1926 ([2, p. 393]).

Two objects of research

Thus far we have always spoken of "the plant" as the object of research (not meaning the individual, but the abstract *Sippe*), in the first instance the species, but also the genus, family, etc., all of which are entities recognized in the *Sippen* of taxonomy, forming the flora. The other object of botanical research is a sociological one, the study of the plant community, which is composed of quasi-organisms that follow their own laws. Under given circumstances of climate, soil, or competition certain groups of plants are always found living together, forming a well defined object of research. This research is plant sociology or the study of vegetation. The term plant sociology is so satisfactory, so to the purpose, that it originated independently in the minds of workers in Germany, Russia, Sweden, Switzerland, and the United States (**1, 13**).

In the analysis of the plant community we need to know the habitat, the physiognomy (life-form), and the composition, the flora which characterizes a certain area. Historical causes (genetic factors, immigration) account for the presence of the species in this given area. The habitat of itself is not botanical; the study of habitat becomes botanical when its effect on vegetation is considered. The vegetation is seen through its physiognomy, especially the part resulting from adaptation. External description of landscape is not scientific botany; the student of physiognomy must penetrate into the details, which include the constitutional characters of species and also epharmosis, adaptation to the environmental conditions which form the habitat.

The habitational point of view began with LINNAEUS, and was taken up by SCHOUW, HEER, and FLAHAULT; the physiognomical one dates from WILLDENOW, HUMBOLDT, and GRISEBACH. The combination of the two lines which alone can bring about real sociology began in the fifties and sixties of the past century (**13**),[6] in the works of SENDTNER, LORENZ, KERNER, and GRISEBACH. We find it spendidly carried through in 1895, in WARMING'S (**22**) *Plantesamfund*, literally translated "plant communities."

[6] So I would speak of seventy years of phytosociology and not of twenty-five (ALECHIN **1**). The science of phytosociology is as old as that, only the word is younger.

The combination of the three large geobotanical problems and the two objects of botany may be tabulated as follows.

THE SIX RESEARCH BRANCHES OF GEOBOTANY (OR PLANT ECOLOGY OR PLANT GEOGRAPHY)

		PLANT *Sippe*; FLORA	PLANT COMMUNITY; VEGETATION
Problem of space	Plant geography	Autochorologic geobotany	Chorologic sociology
Problem of habitat	Plant ecology	Autecologic geobotany	Ecologic sociology
Problem of change	Plant history	Autogenetic geobotany	Genetic sociology (study of succession)

Aims of the six branches

Each branch may be studied in different directions by starting from the problem or from the object. Each branch may be connected with any other one and combined for new viewpoints, new knowledge.

A. PLANT GEOGRAPHY OR CHOROLOGY[7]

1. CHOROLOGIC OR GEOGRAPHIC STUDY OF THE FLORA, AUTOCHOROLOGIC GEOBOTANY.—Floristics belong to this branch. Starting from the object, the plant *Sippe*, one studies the distribution of each species, genus, and family, determines its area, and how it migrated to occupy it. Starting from the problem of space one surveys the flora of a given district. From these floras we learn to divide the globe into floral regions, floral provinces, districts, etc.

2. CHOROLOGIC OR GEOGRAPHIC STUDY OF VEGETATION, CHOROLOGIC SOCIOLOGY.—Starting from the object, the distribution of every plant community recognized over the earth must be studied. More often a local area or district is selected, and in the form of a monograph all communities of this district are determined and surveyed. In applied form we consider where different crops may be grown, the distribution of plant communities indicating the possible distribution of crops.

[7] Logically chorology is a better term for the study of distribution, as geography comprises the study of everything (plant distribution, environment, changes) concerning the earth.

B. Plant ecology

3. Ecologic study of flora, autecologic geobotany.—The habitat of the plant is studied, the means of distribution of the plant and its adaptations; its need of light, moisture, temperature, of soil contents, of competition, the H-ion concentration, etc. The result is the success of a plant in this or that habitat, its growth in a certain adapted life form (supplemented by its constitutional forms).

4. Ecologic study of vegetation, synecologic geobotany, ecologic sociology.—What was considered in the ecology of the single plant is considered here for the whole community; that is, its habitat, including climate, soil, competition, action of animals and man. Large parts of political economy belong in this branch. Forestry is the applied ecology of the plant communities called forests, and in agronomy applied ecology teaches the habitat for corn and fodder growing, etc.

C. Plant history

5. Genetic study of flora, autogenetic geobotany.—In the problem of change of the plant we study the phylogeny of species, and combined with the problem of space how the new *Sippen* created by evolution spread into their area. Starting from the changes of the earth the endeavor is made to follow the development of the flora through the geological periods, some species dying out and others newly coming into existence. The history of every flora and the evolution of species are two great questions of this branch.

6. Genetic study of vegetation, succession of vegetation, syngenetic geobotany.—We study how the plant community comes into existence and how it changes into another one. If the factors which bring about the change are of biotic nature we speak of biotic successions (Cowles); if it is erosion or alluvium we call them topographic successions; when they are conditioned by change of climate of different geological periods they are regional (climatic) successions. Starting from the changing periods of the earth and combining with space and habitat, we study the plant communities of the Tertiary, the Pleistocene, etc.

Combining our branches, nos. 1, 3, and 5 form the study of the flora, all geobotanical problems of the single plant or *Sippe*, the

autogeobotany. Nos. 2, 4, and 6 form the syngeobotany or phytosociology.

To know the plant communities we need to study, besides the main geobotanical problems, the community itself: its morphology determined by its floristic composition, abundance, constancy, and fidelity (and various other minor features) of each constituent, etc. (RÜBEL **14, 15**). Of course a taxonomy of communities is necessary also, and in the ultimate result a system, because we cannot be satisfied by juxtaposition of many results without erecting a philosophical structure of the whole.

History of terms

Having displayed the logical branches of our science and their aims, a few terms must be followed up historically to show how narrower and wider senses arose which led to misunderstandings.

As mentioned, "plant geography" was for a long time and still is the term widely used for comprising our whole science in question, so far as known. With its development it had to be subdivided. Three sections have long been recognized. WILLDENOW (1792) in his history of plants recognized one entity, HUMBOLDT (1793) in a footnote for the first time says that *Geographia plantarum* must be carefully distinguished from *Historia plantarum*. SCHOUW (1822) definitely speaks of plant geography as dealing with present conditions of plants on the surface of the earth, plant history as considering the origin and changes of plants, and plant physiology as the field from which must be learned the action of the external factors on plants. Later on the three fields were generally named floristic (chorologic) plant geography, ecologic (formerly physiologic) plant geography, and historic (evolutional) or epiontologic plant geography.

Basing on physiognomy (growth forms—life forms), and especially on ecology, the recognition and study of plant communities (vegetation, meant in opposition to flora) grew more and more. Plant communities cannot be separated from their cause, the habitat. The ecological idea lies in the concept of plant community. So when WARMING wrote his *Plantesamfund* (plant communities) he gave it the main title of *Lehrbuch der ökologischen Pflanzengeographie* or

Grundträk af den ökologiske Plantegeografi. Therein he treated all the new science of plant communities, and also the ecology of the single plants, the species. This has long persisted of course, and is still often spoken of as ecological plant geography, including of our six branches nos. 2, 3, 4 and 6. In WARMING'S English edition *ökologische Pflanzengeographie* was translated by the short word ecology. So ecology there meant the four branches just cited, but it even extended to the still wider meaning of all six branches.

How did "ecology" come into its position? In plant geography in the first instance geographic, floristic questions were treated. Later on, when ecological points of view deepened the study considerably, not all workers were quick in taking up these new viewpoints. In order to emphasize the deepening by ecological studies, the British vegetation committee laid stress on the term ecology in opposition to mere descriptive floristics. It soon was accepted in the English language for a whole group of geobotanical research branches; many took it for all six (the study of flora and of vegetation); some, according to WARMING'S translation, for the four branches mentioned above; others even for the three branches of the study of vegetation.[8] I prefer to use it only for the two branches of autecological geobotany and synecological geobotany or ecological plant sociology. TAYLOR (**19**) is right when he objects to using ecology without botanical prefix when only botanical questions are dealt with and not zoological ones as well. We never omit "plant" before geography, but often omit "plant" before ecology. Historically this is easily understood. Geography was first used for physical and political geography, and these are cited as geography without prefix. Sometime later the botanical branch of geography was added, the new branch characterized by the prefix "plant." In ecology it was the reverse. The word came into general use through the English and American botanists and WARMING'S translation, so this was used without prefix. Later on animal ecology began to become

[8] COOPER (**2**) p. 395: "Ecology is the science which treats of the vegetation of the earth." Citing this page of COOPER'S most interesting paper, I may refer to an omission. COOPER says about works on succession on the European continent: "Two papers have appeared which deal exclusively with succession, namely, by LÜDI (1919) and FURRER (1922)." Six years earlier (1913) SIEGRIST (**18**) published his paper dealing exclusively with the successions of the alluvial forests near the Aare.

prominent also. We must make allowance for this fact and use the term ecology without a prefix only when animals are included as well as plants. I agree with TAYLOR[9] that phytosociologic concepts are just as applicable to animals as to plants. GAMS (4) has taken the same view as urged by TAYLOR and JONES (8), and has included animals as well as plants in his system of communities (*Biocoenosen*), especially in his *Versuch einer Übersicht über die Lebensformen des gesamten Pflanzen- und Tierreichs* (*l.c.* p. 339).

After the International Botanical Congress of Paris in 1900 had asked authors to make propositions for phytogeographical terminology, SCHRÖTER (16) proposed for the study of plant communities the term *Formationslehre* or synecology, as the study of the plants which dwell together. The field of synecology he divided into physiognomical synecology, geographical synecology, ecological synecology, and historical (*florengeschichtliche*) synecology. Ecological synecology shows the word ecology in the adjectival form for the research problem; in the substantive form for the object of research. Accordingly, autecology, the ecology of the single plant, does not correspond with synecology, the whole study of communities, but only with ecologic synecology, the ecology of plant communities. For the time being then, SCHRÖTER'S term synecology was certainly excellent and served the purpose of advancing the science. But since then this study has deepened considerably and spread widely within all its problems of space, habitat, and change, so as to make it more advantageous to keep synecology for the habitat problem of vegetation, and call the whole science of plant communities, as said before, plant sociology.

Summary

Logically we have in geobotany three great problems of research: space, habitat, and change; and two objects of study: the plant and the plant community. That gives us six branches of our science: (1) autochorologic geobotany; (2) autecologic geobotany; (3) autogenetic geobotany (combining with the study of the flora); and (4) synchorologic geobotany or chorologic sociology; (5) synecologic geo-

[9] I am glad also that TAYLOR emphasizes the great importance of biotic factors; BROCKMANN-JEROSCH and I have always done this as much as possible in most of our papers.

botany or ecologic sociology; (6) syngenetic geobotany or genetic sociology, study of succession (combining with the study of vegetation or plant sociology).

Historically plant geography, plant ecology, and geobotany are synonymous and include all six branches. Geobotany (GRISEBACH) always does this; the two other terms are ambiguous, because often used in narrower and wider senses. The different meanings are discussed.

ZÜRICHBERGSTRASSE 30
ZÜRICH, SWITZERLAND

[*Accepted for publication July 2, 1927*]

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CONTROL OF SEX REVERSAL IN THE TASSEL OF INDIAN CORN[1]

JOHN H. SCHAFFNER

(WITH THREE FIGURES)

Zea mays is an example of the more extreme type of monoeciousness exhibited by various species of angiosperms, since normally the whole inflorescence and a considerable part of the stem and leaf structures below both the ear and the tassel show an evident secondary, sexual dimorphism. It is, therefore, a very favorable species for the study of the influence of sexual states in determining sex-limited morphological characters. Because its monoecious condition is apparently of recent origin, phylogenetically considered, it is also a favorable plant for the study of the control of the time of sex determination in relation to the ontogeny. Theoretically it would appear that the dioecious plants should be the type in which sex reversal could be brought about most readily, because in these plants there is the longest possible period in which to influence or control the various ontogenetic gradients present in the developing system.

Having found, by various experiments and observations on dioecious plants (SCHAFFNER **6, 7, 8, 9, 10**), that the sexual states were determined by functional gradients rather than by differential heredities, and that in some of them sex reversal could be easily induced by simply changing their functional states through the influence of some external environment, and having also studied a number of monoecious species (**7, 10**) to determine the relation of their functional gradients to sex determination, the writer decided to find how much change could be produced directly in Indian corn through an attempted control of its sexual states, its points of sex determination, and its possible sex reversals. In a monoecious plant like corn, the disturbance of the metabolism, with probable consequent changes in the carbohydrate-nitrogen ratio (GARDNER **2**), and other such conditions in the cell complex resulting in changed sexual

[1] Papers from the Department of Botany, The Ohio State University, no. 205.

states at the given points of the determinate growth gradients, should be nearly as easily produced as in dioecious species. This is obvious since the cell development and cell lineage, from the beginning of sex determination in the stem to the production of spikelets or flowers, involves a considerable part of the ontogenetic cycle. To cause a disturbance or a change in the sexual conditions of a plant with bisporangiate flowers of the more extreme type, with its greatly shortened floral axis, in which the determinate gradient is completed, accompanied by the passage from a neutral state to a male or to a female state, would presumably be enormously more difficult because of the great difficulty involved in getting any control of the functional processes in the minute length of axis which is developed before the gradient ends in complete determination. GARDNER (2) was able to induce changes in the sex condition of the Senator Dunlap strawberry, which has bisporangiate flowers. The strawberry belongs to a dioecious group, however, and the Senator Dunlap must be considered as having evolved to the point of dioeciousness or returned to the bisporangiate condition through a recent mutation.

Many monoecious plants which would otherwise be favorable as experimental material are difficult to manipulate because of their geophilous habit. One difficulty with Indian corn is that the inflorescences are completely covered with sheaths or husks until the flowers reach maturity. *Sagittaria*, which might otherwise be very suitable for experimentation, is hydrophytic and thus requires special greenhouse facilities. Corn is on the whole easily cultivated, and because it is greatly influenced by the ordinary environmental factors, it was thought that it ought to be a favorable object for simple experimentation, and that there should be no difficulty in influencing its functional gradients which have their play during the normal ontogeny of the plant. Since sex reversal occasionally occurs in the tassel under ordinary field conditions, apparently the easiest changes would be the reversal of the tassel to partial or complete femaleness, and the shifting of the point of female determination up and down the main stem, which normally takes place only in the incept of a lateral branch. This was therefore selected as the first point of attack, since it was already known that the short light

period had a decided influence in reducing the size of the plant and its photosynthetic surface.

Preliminary tests were made in the winter of 1925–26. On December 14, 1925, sweet corn of the variety Narrow-grain Evergreen was planted on shallow greenhouse benches in soil abundantly supplied with manure and water. There were 28 plants in the plot, spaced about 6 inches apart. Since corn is a slow-growing plant which is not hastened to maturity by a short photoperiodicity to any appreciable extent, the days were already lengthening considerably when flower production began. Nevertheless 21 of the 28 plants in the plot produced tassels with a greater or less degree of carpellateness, resulting in silk and grain development. Thus under the special conditions, this plot of 28 plants showed 75 per cent of the individuals with sex reversal to femaleness in the tassel, and 25 per cent normal monoecious individuals with the tassels in the pure male state.

Another plot was planted December 17, 1925, with Country Gentleman sweet corn which had been selfed for two years by MARION T. MEYERS of the Farm Crops Department, to whom the writer is indebted for the seed. This plot contained 55 plants. Of these 44, or 80 per cent, showed sex reversal in the tassel, and 11, or 20 per cent, showed pure staminate tassels. The results were thus practically the same for the two varieties. It was evident, therefore, that a decided control of sex expression could be accomplished with the development of a suitable program.

During the summer of 1926, Narrow-grain Evergreen sweet corn was planted in the garden with the ordinary spacing for plants in the field. A large patch was studied for evidence of sex reversal in the tassel, but all the tassels, amounting to several hundred, were found to be pure staminate. Under normal field conditions reversal in the tassel must be quite rare in this variety. The development of silks and grain in the greenhouse plants, therefore, was due to the short light period in combination with the other environmental factors. It was noted that those plants which had sprouted somewhat later than their neighbors, and were thus soon somewhat shaded, not only remained quite small in comparison with the others but also showed the greatest degree of sex reversal.

With this preliminary knowledge of the apparently efficacious environmental factors, an experiment was planned for an attempted complete percentage control of sex reversal in the tassel. A tank 3 feet deep and 3 feet wide was available, and in this Narrow-grain Evergreen sweet corn was planted in well manured soil, November 1, 1926. All of the 20 plants of this plot showed sex reversal in the tassel. There was therefore 100 per cent control, so far as the possibility of reversal was involved. The degree of reversal was of varying

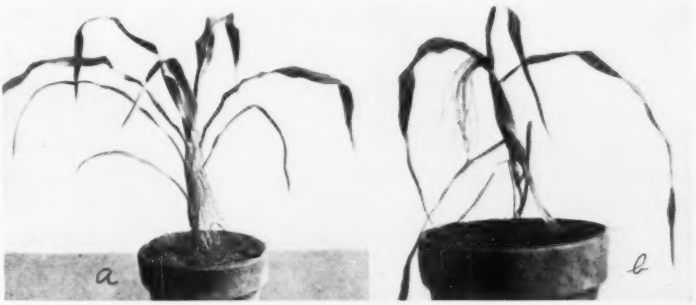

FIG. 1.—*a*, completely reversed plant, showing abundant silks developed from terminal inflorescence and reaching ground, taken before crimping of stem; *b*, extreme type of carpellate plant, showing flexuous condition beginning to appear at second node and forcing leaf sheaths apart; both plants transferred to pots for photographing.

intensity and completeness, ranging all the way from tassels with a few silks and developing grains among the staminate flowers, up to those which were completely reversed and showing a fairly well developed cob with no staminate flowers whatever. The 20 plants began blooming January 25, and the blooming period was completed February 15, when the last plant began to protrude silks from the terminal sheaths. The most extremely carpellate plant was exactly 6 in. high (15 cm.) to the point at which the silks emerged from the leaf sheaths, and had just 6 nodes and leaves (fig. 1 *b*). The taller, less extremely reversed plants had as many as 12 leaves.

On February 14 all the plants except one had been removed from the plot; so it was immediately replanted with Narrow-grain Evergreen sweet corn in order that a second crop might be obtained, developed under the influence of the lengthening days of spring.

There were 25 plants in this second plot, and of these only 3 showed sex reversal to a slight degree, or 12 per cent. Of the 3 plants with reversal in the tassel one had 3 silks and the other two had 2 silks each. The leaves of the plants of this plot ranged from 10 to 15. Evidently the longer light had a decided influence in promoting maleness in the inflorescence.

During the winter several plots of the Narrow-grain Evergreen variety were planted also on shallow benches. These behaved much like the plants of the previous winter, and were less extreme in sex reversal than the plants in the deep soil in the tank. One plot of 17 plants, planted November 2, 1926, showed 13 with reversed tassels, or 76.5 per cent. Another planted November 16 had 11 plants, 8 of which showed reversal, or 72.5 per cent. A third plot, part of which was destroyed by mice, was planted December 17, and of the nine surviving plants five, or 55.5 per cent, showed sex reversal in the tassel. The shallow soil of the benches was apparently less favorable for producing sex reversal than the deep soil in the tank.

Some of the plants from the benches were studied for the relative degree of sex reversal. As indicated, they ranged all the way from no reversal to complete female expression in the tassel (figs. 1 *a*, 2 *a–g*, 3 *a*). A few records are as follows: 1 tassel had 1 silk, 1 had 2, 2 had 3, 2 had 4, 2 had 5, 2 had 6, 1 had 9, 1 had 10, 2 had 15, 1 had 16, 1 had 18, 1 had 19, 2 had 20, 1 had 23, 1 had 28, 1 had 33, 1 had 42, 1 had 50, 1 had 53, 1 had 88, and 1 had 112. The reversal is usually complete and produces a good grain, even when only a single flower is affected and a single silk developed in the entire tassel. These silks may be in any position in the tassel or its branches, from the bottom to the top. Thus a well developed tassel having 5 silks had 2 of them near the base, 2 above the middle, and 1 within half an inch of the tip. More commonly the sex reversal is mainly at or near the base of the inflorescence.

A summary of all the plantings is shown in table I. It is evident that with uniform and favorable conditions the degree of sex reversal varies with the relative length of daylight, very much as the writer found true for hemp (**6, 9**).

Although the vegetative period of development seems little changed by the short photoperiodicity, the reduction of the number

FIG. 2.—Series of plants taken from plot on shallow benches, showing gradation from normal monoecious condition to rather extreme carpellate development; leaves removed to show flexuous condition of the partially reversed plants: *a*, *b*, normal monoecious condition; *c–g*, various degrees of sex reversal in tassel.

of nodes and leaves is very marked. One of the most striking changes is produced in the main stem. With the change of functional gradient and in the extreme conditions of reversal, femaleness is mostly determined some distance below the tassel. In the most extreme cases, the secondary female state was induced in the second or third nodes from the base of the stem (figs. 1 *b*, 3 *a*). Since the

FIG. 3.—*a*, extreme reversal condition, showing complete female expression; *b*, two plants with leaves removed, both showing decided sex reversal in tassels (but still somewhat branched); femaleness determined in main shoot just above uppermost ear-producing node, consequently terminal stems decidedly flexuous.

secondary female state which develops in the incepts of the lateral or ear buds throws the leaf sheaths into the husk condition, and also causes a flexuous development of the shank or ear stem, especially when this is somewhat elongated, one would expect a somewhat similar action in case femaleness is developed in the main stem. In the more extreme cases the sheaths are decidedly influenced, showing considerable husk characteristics, and the stem becomes remarkably flexed, in some cases even developing double loops when the internodes are long (fig. 2 *e*). The flexuous condition is well shown

in figs. 1 *b*, 2 *c–g*, and 3 *b*. Fig. 2 *a* and *b* show the normal straight stems of normal monoecious plants in which no sex reversal has taken place. Fig. 2 *c* has a slight double kink in the first node below the tassel which showed a partial sex reversal. In figs. 1 *b* and 3 *a* the flexuous condition is shown in one or more nodes below the first ear bud. These stems are at first perfectly straight, but as lengthening of the internodes proceeds the one-sided development becomes very

TABLE I

SUMMARY OF PLANTINGS SHOWING PERCENTAGE OF REVERSAL IN TASSEL

DATE OF PLANTING	TOTAL NO. OF PLANTS	REVERSED TASSELS	NOT REVERSED	PERCENTAGE OF REVERSAL	VEGETATIVE PERIOD (DAYS)
1925–26					
December 14	28	21	7	75	.....
December 17*	55	44	11	80	.....
1926–27					
November 1†	20	20	0	100	75
November 2	17	13	4	76.5	.....
November 16	11	8	3	72.5	.....
December 17	9	5	4	55.5	.....
February 14†	25	3	22	12	78
March 15	27	1	26	3.6	72

* Variety in this plot was Country Gentleman sweet corn.

† Planted in deep soil in tank.

prominent, forcing the sheaths apart (fig. 1 *b*), and often causing the tip of the plant to be bent toward the ground when the support given by the sheaths is lost. These extreme plants with their flexuous stems and terminal silks present a very bizarre appearance when compared with corn plants with the normal hereditary expression. Such plants can now be produced easily at any time for demonstration purposes with the proper environmental control.

The tassels showing sex reversal are usually only slightly branched. This is the usual effect of the presence of the female state, although the tassels without sex reversal developed in a shorter light period than the normal are also only slightly branched and occasionally not at all.

The reversal of the tassel to the carpellate condition in the two varieties of sweet corn tested is to be regarded as an ecological fluctuation depending on a complex of environmental factors. With the

proper moisture, nutrient supply, temperature, and dimness of light, the amount of reversal, both in respect to the population as a whole and the degree to which it is expressed in any individual, is proportional to the shortness of the daily illumination period. Since intensity of illumination seems to have a decided influence, proper spacing is also important if one is attempting to obtain uniform reversal ratios. Perhaps the percentage of reversal will also be found proportional to the degree of light of low intensity, if the photoperiodicity is kept constant.

Summary

1. Narrow-grain Evergreen sweet corn is decidedly influenced in its sexual expression by the length of the daily illumination period, and the amount and degree of reversal to femaleness in the tassel is subject to experimental control. Limited experiments with Country Gentleman sweet corn indicate that the same condition is present in that variety.

2. Corn planted on November 1 in the greenhouse will, with proper substratum and heat, show 100 per cent of the individuals with some degree of female expression in the tassel, while that planted in the spring or summer will normally show only pure staminate tassels.

3. Corn planted either before or after this date, under similar conditions, will show a ratio of sex reversal inversely proportional to the length of the daylight. With the approach of equal day and night periods of 12 hours each, little or no reversal will occur in the tassel.

4. The time and point of female sex determination can be thrown back to the second or third leaf nodes from the base of the plant.

5. Whenever the female state is established in the main stem, either above or below the point of lateral ear development, the internodes become decidedly flexuous, and in extreme cases they are even thrown into loops.

6. Any plot of corn, grown in the greenhouse, can be so planted and developed that the individuals of the population will show every gradation, from the normal monoecious type, with pure carpellate-

ness expressed in the side branches and pure staminateness in the terminal inflorescence, to an absolutely pure female condition, all the flowers of the terminal inflorescence as well as those of the lateral ears being purely carpellate.

OHIO STATE UNIVERSITY
COLUMBUS, OHIO

[*Accepted for publication June 16, 1927*]

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CURRENT LITERATURE

BOOK REVIEWS

Flowering plants

The second volume of RENDLE'S[1] presentation of the classification of flowering plants has appeared twenty years after the publication of the first volume. The author states that "increasing official and extra-official duties and responsibilities have allowed insufficient leisure for the continuous effort required." This delay has resulted in the advantage of his being able to include the recent advances in our knowledge of dicotyledons and their relationships.

The general arrangement follows that of ENGLER'S *Syllabus der Pflanzenfamilien*, and the nomenclature is that adopted by the Vienna Congress of 1905. Three grades are recognized in the phylogeny of dicotyledons, Monochlamydeae, Dialypetalae, and Sympetalae. The author does not claim that the sequence is strictly phylogenetic. He states that "various attempts have been made to construct a phylogenetic system of angiosperms, but the results are not convincing, bear no suggestion of permanence, and bristle with difficulties for the student."

The three grades used correspond to grades of differentiation in the floral structure. The Monochlamydeae include the orders with a comparatively simple type of flower. Several of them represent very isolated groups. This grade extends from the Salicales to the Centrospermae, in the latter order the higher type of floral structure existing parallel with the simple monochlamydeous type. In the Dialypetalae, extending from the Ranales to the Umbelliflorae, the orders are arranged in ascending sequence, from indefinite to definite numbers of floral members, from spiral to cyclic arrangement, from hypogyny to epigyny, and from regularity to zygomorphy. The Sympetalae represent still higher grades of floral complexity, extending from the Ericales to the Campanulales. The evidence is that this culminating group of the plant kingdom has arisen from various groups of the Dialypetalae.

Each family is presented in great detail and well illustrated. Not only are the distinctive characters given, but also a full account of suggestive relationships, geographical distribution, life habits, cultivation, and uses. In short, the purpose seems to be to present every known aspect of the family, so that one may become fully acquainted with it in its setting in the plant kingdom. In a

[1] RENDLE, A. B., The classification of flowering plants. Vol. II. Dicotyledons. 8vo. pp. xix+636. *figs. 279.* 1925. London: Cambridge University Press; New York: Macmillan Co.

book which is such a thesaurus of information it is impossible for a brief review to select any details. The volume will certainly prove to be an invaluable help, not only to students of classification, but also to all botanists.—J. M. C.

Genetics in relation to agriculture

The second edition of the volume by BABCOCK and CLAUSEN,[2] which the authors promised us several years ago, has just appeared. This delay in publication has been well worth while, permitting the inclusion of numerous very significant investigations of the past few years. So conscientiously have the authors attempted to keep pace with this most rapidly moving of all subjects, that the present text bears only a superficial resemblance to its predecessor of nine years ago.

In total pages the two editions are practically the same. There has been, however, a considerable enlargement of the material on fundamental genetics (Part I), with a compensating reduction of the practical applications to plant and animal breeding (Parts II and III).

The fourteen chapters which comprised Part I of the earlier edition are replaced by twenty-seven shorter chapters, a device which makes the book much more readable, particularly for the undergraduate. The sequence of subject matter in this part is thoroughly recast. A much more substantial foundation is laid by the thirty-one pages on "Physical basis of heredity" than the eleven pages which were there before. Following this, chapters are grouped under the two general headings of Heredity and Variation, in the sequence indicated, which is a worth while reversal of the sequence in the earlier volume.

Of the twelve chapters on Heredity, three are very largely new, comprising two short chapters on Lethal factors and Sex limited characters, and a long one on Organization of the linkage groups. This last chapter, like the handbook for an automobile, makes the machine more than a mere convenience, and actually tells us how to take it apart and put it together again.

The general section on Variation contains a much more refined analysis of mutation than before. Instead of one chapter on the subject there are now five. Factor mutations (Chapter 22), Chromosomal variation (Chapter 24), and Oenothera investigations (Chapter 26) might have been expected. Chapters 23 and 25, however, are a delightful surprise. The former, on Parallel variation, devotes thirteen pages to the very suggestive problem of factor mutations of independent origin in related species. The authors have wisely set apart from Chromosomal variation a special subject which merits an identity of its own. In Chapter 25, under the title Sectional variation, they discuss the instances where a portion of a chromosome has become lost or translocated.

After reading the text, one is not surprised to find thirty-five pages devoted to the list of literature (approximately a thousand titles). The general biological

[2] BABCOCK, E. B., and CLAUSEN, R. E., Genetics in relation to agriculture. pp. xiv+673. *pls. 4. figs. 203.* New York: McGraw-Hill Book Co. 1927. $5.00.

public, as well as the authors, are to be congratulated upon the appearance of this volume.—M. C. COULTER.

Fungi

GWYNNE-VAUGHAN and BARNES[3] have published a much needed textbook, presenting the fungi to students rather than to investigators. It includes all the fungi, so that the student may obtain a general perspective of this very complex assemblage. Pathology is not included, since the authors state that they "are concerned primarily with the fungus, and only secondarily with its effect on other organisms."

The organization of the textbook is suggestive. It begins with a general account of the structure of fungi, including the Myxomycetes and Plasmidiophoraceae, "forms resembling fungi." The physiology of fungi is presented under the following headings: saprophytism, parasitism, symbiosis, specialization, and reaction to stimuli. Twelve major groups are then presented, from Phycomycetes to Autobasidiomycetes, followed by an account of the Fungi Imperfecti. These groups are fully described and well illustrated. The descriptions are not in technical style, but in language that will be understood by the student. The book closes with a very effective description of mycological technique, so that the student may know how fungi are investigated.

This book will prove to be a very effective introduction to fungi for students, the presentation being so well adapted to their needs that they will not only be instructed, but also interested.—J. M. C.

Soil mineralogy

A useful little handbook on soil mineralogy has been prepared by BURT,[4] and those who want a more intimate knowledge of the species of minerals found in their local soils will find it a valuable aid. The first section gives in three brief chapters some general information regarding the physical properties of soil minerals, such as color, streak, luster, tenacity, hardness, cleavage, fracture, specific gravity, fusibility, form, and structure; the elements actually occurring in soils, including twenty-one of the more commonly found elements; and the processes of weathering, which are considered under the headings hydration, carbonation, oxidation, hydrolysis, reduction, sulphation, and chloridation.

The second part presents a tabulated view of the analysis of minerals by blowpipe methods. The key suffices for the determination of somewhat more than a hundred of the commoner minerals of the soil. The third section describes about sixty-five minerals in ten classes, halides, sulphides, oxides, hydroxides and hydrous oxides, silicates, carbonates, sulphates, phosphates, borates, and nitrates, and metaferrite and metatitanite. The fourth part consists of four

[3] GWYNNE-VAUGHAN, H. C. I., and BARNES, B., The structure and development of the fungi. London: Cambridge University Press. 8vo. pp. xvi+384. *figs. 285.* 1927.

[4] BURT, F. A., Soil mineralogy. 8vo. pp. viii+81. New York: Van Nostrand. 1927.

tables showing the general occurrence of soil minerals, their relative weathering resistance, the volume changes involved in weathering changes, and the physical properties of twenty-one of the commoner soil constituents.

The brevity and conciseness of the book appeal to the student. In many cases one would want to consult the larger works after having exhausted the information obtainable from use of this volume, but it should be a welcome aid to everyone who uses soils in connection with the investigation of plant life.—C. A. SHULL.

Hydrogen ion concentration

A recent translation[5] of MICHAELIS's *Wasserstoffionenkonzentration* needs no description for American students of plant physiology. The earlier German editions have been used very widely in this country, but it will be more widely available and still more widely used in this translation by Professor PERLZWEIG.

Some of the more recent advances in this field have been added by MICHAELIS, so that it is more valuable even than the second German edition. These additions concern the newer contributions to the activity theory of ionization, recent modification of the theories of dissociation of ampholytes, and the theories of oxidation-reduction potentials, including use of the quinhydrone electrode.

Students will find it very worth while to study the volume carefully. The first five chapters lay down the general principles of physical chemistry now so much needed by biological investigators, and the last five deal with ions as sources of electrical potential differences. An important service has been rendered in the translation, which has been exceedingly well done. In its appearance as a book, it lives up to the excellent traditions of the publishers.—C. A. SHULL.

Flora of Jamaica

In continuation of their *Flora of Jamaica*, FAWCETT and RENDLE[6] have published a volume describing the dicotyledons from Buxaceae to Umbelliferae, completing what they call the "free-petaled" dicotyledons. The volume is thoroughly organized, with analytical keys, full descriptions, excellent illustrations, complete synonymy and literature, and geographical distribution. It includes 42 families, represented by 158 genera and 467 species. In the main the genera are represented by very few species, no fewer than 80 of them being monotypic, so far as Jamaica is concerned. Much the largest families are the Malvaceae (69 species), Melastomaceae (69 species), and Myrtaceae (60 species). The largest genus is *Eugenia* (Myrtaceae) with 37 species, the next in size being *Miconia* (Milastomaceae) and *Sida* (Malvaceae), each with 19 species.

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[5] MICHAELIS, LEONOR, Hydrogen ion concentration, its significance in the biological sciences and methods for its determinations. Transl. by WM. A. PERLZWEIG. 8vo. pp. xvi+299. Baltimore: Williams and Wilkins.

[6] FAWCETT, W., and RENDLE, A. B., Flora of Jamaica. Vol. V, Part III. 8vo. pp. xxviii+453. *figs. 156*. British Museum Publ. 1926.

Most of the genera are represented by only one or two species. The contrasts with the flora of the United States are often very striking. For example, the Umbelliferae are represented in Jamaica by only 6 species, representing 3 genera. Many such contrasts occur, and are very suggestive to students of geographical distribution.—J. M. C.

Fungi of Middle Europe

Numbers 3 and 4 of volume I of *Fungi of Middle Europe*[7] have made their appearance. The general scheme of this new work on fungi has been reviewed previously.[8] Number 3 carries a discussion of *Boletus rhodoxanthus*, *B. impolitus*, *B. pseudosulphurens*, and *B. pulverulentes*, with two colored plates of the last two species, and two plates in black and white illustrating habit and structural features of these and of *B. rimosus*, *B. parasiticus*, and *B. regius*. Part IV consists of a discussion of *B. rimosus* and *B. erythropus*, and of two colored plates of these organisms.—G. K. K. LINK.

Zoocecidia of Netherlands East Indies

A comprehensive work has been published on the animal galls of the Dutch East Indies.[9] The first nine chapters are devoted to general topics, such as collection of gall producers, geographical distribution of galls, and phylogenetic considerations (H. H. KARNY). Chapter X, which comprises the major part of the text, gives descriptions of galls, beginning with those of the Hymenophyllaceae and ending with those of the Compositae. There are seven plates (four in color), and bibliographical, host, and pathogen indices.—G. K. K. LINK.

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[7] Die Pilze Mitteleuropas, under the editorship of KNIEP, H. (Berlin), CLAUSSEN, P. (Marburg), and BASZ, J. (Stuttgart). Leipzig: W. KLINGHARDT. Band I. No. 1. 1926. M. 4.00 per number.

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